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VOL. 25 NUMBER 4

PLANT PHYSIOLOGY

UNIVERSITY OF MICHIGAN
NOV 7 1950

OCTOBER, 1950

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PUBLISHED QUARTERLY
BY
THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS
AT E. QUEEN ST. AND McGOVERN AVE., LANCASTER, PA.
BUSINESS PRESS, INC.

Entered as second-class matter March 25, 1926, at the post office at Lancaster, Pa., under the Act of August 24, 1912.
Accepted for mailing at the special rate of postage provided for in paragraph 4, section 538. Authorized April 9, 1926.

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A QUARTERLY JOURNAL
DEVOTED TO ALL PHASES OF PLANT PHYSIOLOGY

Established 1926

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Subscription price, $10.00 a year. Single copies, $3.00 post free. Members of the American Society of Plant Physiologists receive the journal in connection with dues paid to the Society.

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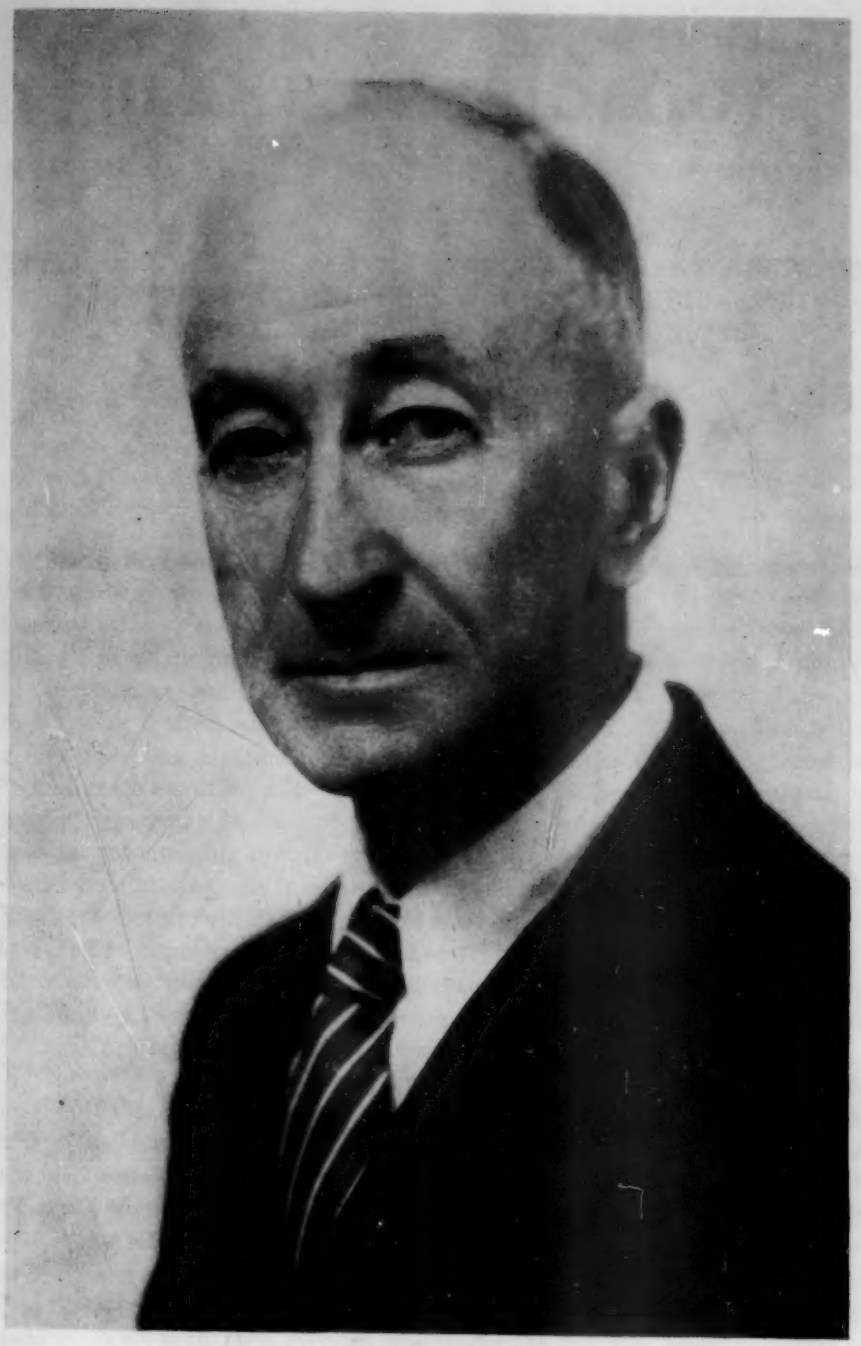

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PLANT PHYSIOLOGY

VOLUME 25 OCTOBER 1950 NUMBER 4

THE EFFECT OF PREHARVEST FOLIAGE SPRAYS OF CERTAIN GROWTH REGULATORS ON SPROUT INHIBITION AND STORAGE QUALITY OF CARROTS AND ONIONS[1]

S. H. Wittwer, R. C. Sharma, L. E. Weller and H. M. Sell

(WITH THREE FIGURES)

Received May 18, 1950

The methyl ester of alpha-naphthaleneacetic acid has been recommended for inhibiting storage sprouting of table stock potatoes. Several formulations of it have been developed for commercial use. However, attendant difficulties, particularly as to satisfactory and convenient methods of applying the chemical inhibitor, have persisted. Failures have resulted if the potatoes were treated when no longer dormant (**2**). The tubers of some varieties following treatment have developed callus-like types of outgrowths in bud areas resulting in severe cracking and proliferations (**10**). Little is known concerning the use of the methyl ester of alpha-naphthaleneacetic acid for maintaining dormancy in stored root crops such as carrots, beets, turnips, rutabagas, and parsnips, although it has generally been conceded that both root growth and top growth are reduced by treatment and that the storage life is prolonged (**9**). Apparently the methyl ester of alpha-naphthaleneacetic acid has not been used successfully for sprout prevention on the onion, a possible explanation being that the vapors do not penetrate to the well protected and enclosed growing points.

The problems of retarding growth of roots and sprouts on root crops and bulbs and of maintaining dormancy in potato tubers by chemical treatment would be greatly simplified if some growth substance could be sprayed on the foliage while the plants were still growing in the field. Using this method, Ellison and Smith (**4**) have reported considerable sprout inhibition on potatoes with 2,4,5-trichlorophenoxyacetic acid. However, unless the chemical was applied late in the season, yields were reduced, the plants were injured, and a scabby condition developed on the tubers. This scab formation on the tubers has recently been identified as common scab (*Streptomyces scabies*), and its development is apparently stimulated by the application of a growth regulator (**13**). Since most

[1] Journal Article, No. 1158 of the Michigan Agricultural Experiment Station.

growth substances are readily translocated downward in the plant, foliage applications might be successful not only with root crops such as carrots but with the onion as well, thus permitting the intact growing plant to translocate the inhibitory substance to the growing points. Such a means of inhibiting sprout and root growth would be an aid in prolonging the storage life and quality of many vegetables in the northern states. This report summarizes the results obtained with respect to sprout retardation, keeping quality, and chemical composition of stored carrots and onions when preharvest sprays of several growth regulators were applied to the foliage of the crops.

Preliminary studies

During the summer and fall of 1948, the sodium salt of alpha-naphthaleneacetic acid and 2,4,5-trichlorophenoxyacetic acid were applied separately as sprays at intervals during the growing season to the foliage of Nantes, Danvers and Chantenay carrots and yellow Sweet Spanish onions. Based on reports for potatoes (**4**), water spray concentrations ranged from 500 to 5000 parts per million (p.p.m.) for the sodium salt of alpha-naphthaleneacetic acid, and from 25 to 250 p.p.m. for 2,4,5-trichlorophenoxyacetic acid. At weekly intervals following the spray treatments, 15-pound lots of carrots and onions were harvested and placed in storages of various temperatures to observe sprouting, root growth, and keeping quality. Two control comparisons were used: (a) non-treated lots, and (b) post-harvest application in commercial dust form of the methyl ester of alpha-naphthaleneacetic acid equivalent to 0.9 grams active ingredient per bushel.[2]

With onions all chemical treatments, including the post-harvest application of methyl ester of alpha-naphthaleneacetic acid, tended to stimulate rather than inhibit sprout growth and rooting. All treatments shortened rather than prolonged storage life. An abnormality commonly termed "blowout" developed on the bases of many bulbs. The "blowout" condition apparently resulted from an elongation of the bases of the leaf sheaths induced by the greater growth activity of the buds. Leaf bases in the elongated and enlarged region were much thinner, having been stretched, than in the sheaths not affected (fig. 1). Early applications of the higher concentrations of both chemicals, in particular, stimulated sprouting, the production of "blowouts," and resulted in earlier and more storage breakdown.

Somewhat similar results were obtained on carrots with the various 2,4,5-trichlorophenoxyacetic acid treatments. There was no retardation of sprouting. Rooting and callus formations were stimulated in the numerous areas formed by the expanded openings through which the lateral roots had appeared. The effect of the treatment was not only to produce callus tissue in which new adventitious roots appeared (hypocotyl) but its effect was also evident in the development of new lateral roots. Such con-

[2] Formulated as "Barsprout" by the American Cyanamid Company, New York.

ditions detracted from the general appearance of the carrots, produced more spoilage and greatly shortened the storage life. However, a distinct interaction of these chemically induced effects with variety was noted. Danvers was most severely affected, while Chantenay showed little, if any, injury, Nantes being somewhat intermediate in its response (fig. 1).

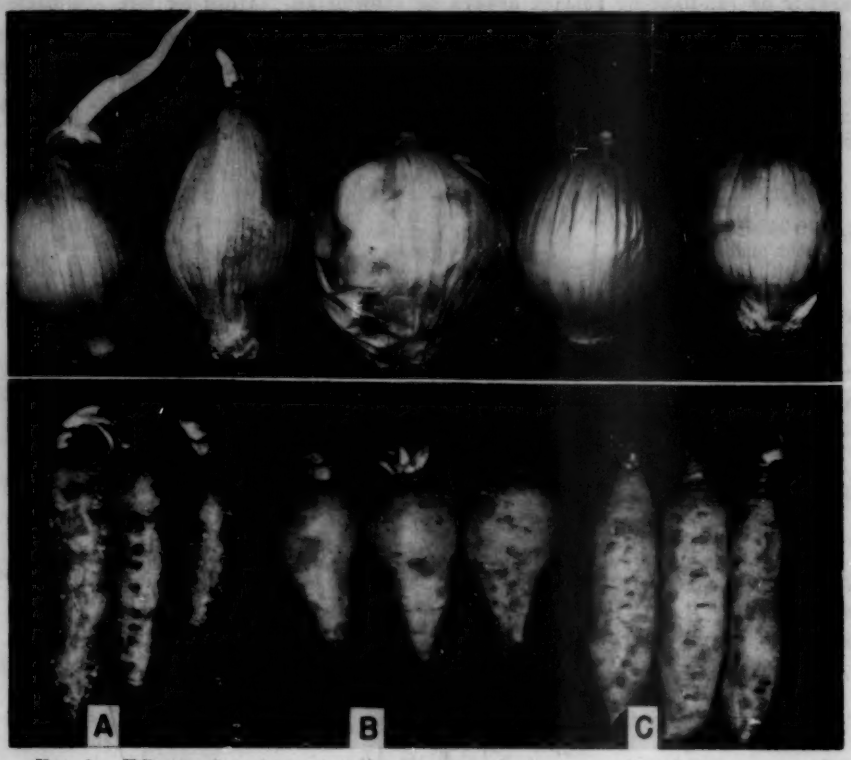

FIG. 1. Effects of preharvest foliage sprays of plant growth substances on onions and carrots: Bottom, variable response of Danvers (A), Chantenay (B), and Nantes (C) carrots to 50 p.p.m. of 2,4,5-trichlorophenoxyacetic acid; Top, the "blowout" abnormality induced by 5000 p.p.m. of the sodium salt of alpha-naphthaleneacetic acid.

Concentrations of 1000 to 5000 p.p.m. of the sodium salt of naphthaleneacetic acid reduced sprouting significantly on all three varieties, but again with this chemical storage life was shortened rather than prolonged because of the accompanying callusing and proliferations in the areas where the lateral roots had appeared. Noticeable callus production was observed in some instances within three to four days following treatment and before the carrots were harvested. This abnormality continued to develop in storage and became progressively more severe. The post-harvest treatment of the methyl ester of naphthaleneacetic acid resulted in reduced sprout production, but stimulated callusing, rooting and rotting to an extent that the storage life was shorter than that of non-treated controls.

Methods and procedure

Detailed tests were conducted in 1949 with several additional chemicals as indicated in tables I and III. The same three carrot varieties of

TABLE I

EFFECT OF PRE-HARVEST FOLIAGE SPRAYS OF GROWTH REGULATORS ON THE SUBSEQUENT SPROUTING OF CARROTS IN STORAGE
(GRAMS OF SPROUTS PER 20 CARROTS AFTER THREE MONTHS' STORAGE AT 50° F.)

TREATMENTS	DANVERS	NANTES	CHANTENAY	MEANS
Sodium salt of alpha-naphthaleneacetic acid				
1000 p.p.m.	16.7	20.2	22.0	19.6
5000 p.p.m.	13.9	7.8	3.8	8.5
2,4,5-trichlorophenoxy-acetic acid				
10 p.p.m.	31.8	33.6	27.5	30.9
50 p.p.m.	25.6	12.5	20.0	19.3
Alpha-o-chlorophenoxy-propionic acid				
25 p.p.m.	46.4	34.7	12.9	31.3
100 p.p.m.	42.6	24.4	13.5	26.8
Benzothiazol-2-oxyacetic acid				
500 p.p.m.	22.0	10.0	2.5	11.5
2500 p.p.m.	2.3	0.3	0.0	0.9
Maleic hydrazide				
100 p.p.m.	53.2	31.1	23.0	35.7
500 p.p.m.	45.2	25.3	13.2	27.8
2500 p.p.m.	2.8	6.4	0.8	3.3
No treatment—Controls	54.0	37.9	24.6	38.8
Means	29.7	20.4	13.7	

Differences Necessary for Significance	5% Level	1% Level
Between Treatments	10.3	13.8
Between Varieties	8.2	18.8

TABLE II

THE EFFECT OF PREHARVEST FOLIAGE SPRAYS OF MALEIC HYDRAZIDE, AS COMPARED WITH NON-TREATED CONTROLS, ON THE NUTRIENT COMPOSITION OF THREE VARIETIES OF CARROTS AFTER THREE MONTHS' STORAGE AT 50° F.
(VALUES REPRESENT AVERAGES OF TWO 10-CARROT LOTS SAMPLED FROM REPLICATED FIELD PLOTS AND ARE EXPRESSED AS PERCENTAGES OF DRY WEIGHT)

PLANT CONSTITUENT	DANVERS		NANTES		CHANTENAY	
	CONTROLS	MALEIC HYDRAZIDE	CONTROLS	MALEIC HYDRAZIDE	CONTROLS	MALEIC HYDRAZIDE
Dry Matter	14.7	13.9	12.0	12.3	12.8	12.5
Carotene*	738	777	802	779	1051	1221
Reducing Sugar	18.1	18.4	26.9	25.5	21.5	20.0
Non-Reducing Sugar	15.3	16.3	11.6	9.7	10.9	12.3
Starch	1.9	2.5	2.6	2.2	1.7	1.4
Polysaccharides**	8.0	6.2	7.5	8.1	5.9	6.3
Total Carbohydrates	42.3	43.4	48.6	45.5	40.0	40.0
Kjeldahl Nitrogen	1.77	2.23	1.71	1.83	2.12	2.55

* Expressed as parts per million (dry weight basis).
** Acid hydrolyzable residue from starch determinations.

numbered stock seed[3] and the yellow Sweet Spanish onion (Joseph Harris Seed Co.) were grown. The carrot seed was planted June 20 on mineral soil. Water solutions of the various growth substances (Table I) were applied as sprays to the foliage of two 20-foot row replicates of each variety on September 30. Triton B-1956 (supplied by Rohm and Haas Company, Philadelphia, Pennsylvania) at a concentration of 0.1% was used as a wetting agent. The chemical solutions were applied at the rate of 75 gallons per acre by means of three-gallon hand sprayers. The car-

TABLE III

EFFECT OF PREHARVEST FOLIAGE SPRAYS OF GROWTH REGULATORS ON SUBSEQUENT SPROUTING AND BREAKDOWN OF YELLOW SWEET SPANISH ONIONS IN STORAGE

TREATMENTS	WEIGHTS OF SPROUTS (GMS. PER 40 BULBS)	PERCENTAGE OF STORAGE LOSS FROM	
		SPROUTING	BREAKDOWN
Sodium salt of alpha-naphthaleneacetic acid			
1000 p.p.m.	64.7	23	38
5000 p.p.m.	36.8	22	40
2,4,5-trichlorophenoxy-acetic acid			
10 p.p.m.	67.4	23	35
50 p.p.m.	68.1	28	46
Sodium salt of beta-naphthoxyacetic acid			
500 p.p.m.	22.2	19	48
2500 p.p.m.	45.3	14	49
Benzo-thiazol-2-oxyacetic acid			
500 p.p.m.	40.4	20	48
2500 p.p.m.	53.6	30	44
Maleic hydrazide			
100 p.p.m.	31.7	26	40
500 p.p.m.	3.9	10	21
2500 p.p.m.	0.0	0	15
"Barspout"—Controls	28.8	26	69
No treatment—Controls	40.5	19	26
Difference Necessary for Significance Between Treatments			
5% Level	24.4	13	23
1% Level	34.3	18	33

rots were harvested four days following treatments, cleaned, placed in replicated lots of 20 carrots in kraft paper bags, and held for three months at a temperature of 50° F and a relative humidity of 90–95%. As in the preliminary tests, there were two controls for comparison: (a) non-treated lots, and (b) post-harvest application of "Barsprout."

The Sweet Spanish onions were started from greenhouse grown plants, seeded March 1, and transplanted into a field of productive mineral soil the second week in May. On August 15, when the tops were still green

[3] Danvers, stock No. 7263, Nantes, stock No. 7269, and Chantenay (Hall type), stock No. 4433. All supplied by the Ferry-Morse Seed Company, Detroit.

and approximately one-third of them were down, water solutions of growth substances (table III) were applied to four 20-foot row replicates in a manner similar to that already described for carrots. One week after treating, the remainder of the tops were turned down. The onions were harvested August 29. After the bulbs were cured for two weeks at a temperature of 85° F and a relative humidity of 50 ± 7%, they were placed in replicated lots of 20 bulbs in kraft paper bags and removed to a cold storage room (35° F) for 30 days, following which they were held at a temperature of 55 ± 3° F and a relative humidity ranging from 65 to 85%. As with carrots, the two controls for comparison were included.

Results

CARROTS.—Significant differences, both with varieties and treatments (table I), were observed in sprouting. Sprouting was practically nil at 2500 p.p.m. of either maleic hydrazide[5] or benzo-thiazol-2-oxyacetic acid (Supplied by the American Cyanamid Company, New York). Lower concentrations (500 p.p.m.) of maleic hydrazide and benzo-thiazol-2-oxyacetic acid and the two concentrations of the sodium salt of alpha-naphthaleneacetic acid also significantly retarded sprouting. After three months' storage at 50° F, the carrots which received the post-harvest treatment of the methyl ester of alpha-naphthaleneacetic acid ("Barsprout") were no longer marketable because of excess callus production and spoilage. Accordingly, the data for it are not given. The relative amount of sprouting and the condition of the roots of the 3 varieties as related to selected treatments are illustrated in figure 2. Carrots treated with maleic hydrazide, in addition to being free from sprouts, were clean, smooth and bright showing no evidence of wilting, callusing, or tissue proliferations.

Chemical analyses (**1, 8**) of the twice replicated lots of carrots sampled after three months' storage at 50° F showed no significant differences in dry matter, carotene or carbohydrates, resulting from treatment with 2500 p.p.m. of maleic hydrazide. Varietal differences, however, were apparent (table II). The percentages of Kjeldahl nitrogen were higher, just under the 5% level of significance, in all carrot varieties that received the maleic hydrazide treatment.

ONIONS.—Observations were made March 2, 1950, after the onions had been held in storage for five months. No sprouting was evident on bulbs which had been harvested from plants, the tops of which had been sprayed with 2500 p.p.m. of maleic hydrazide, and there was a significant reduction in sprouting with 500 p.p.m. (table III). Gross longitudinal sections (fig. 3) of bulbs resulting from treatment with 2500 p.p.m. of maleic hydrazide revealed an internal structure that was normal and indistinguishable from non-sprouting controls (not treated). Flavor, color and odor were apparently not affected. Some of the bulbs from the non-treated controls

[4] Formulated as the water soluble diethanolamine salt of 1,2 dihydro 3,6 pyridazinedione, and supplied by the U. S. Rubber Company, Naugatuck Division, Naugatuck, Connecticut.

which had not sprouted by March 2, and bulbs harvested from plants which had received the 500 and 2500 p.p.m. of maleic hydrazide were held for an additional six weeks in storage at 55° F and photographed April 15 (fig. 3). Similar bulbs of the maleic hydrazide (2500 p.p.m.) treatment planted in the greenhouse remained completely dormant for eight weeks, while non-treated bulbs grew normally and produced large vegetative tops.

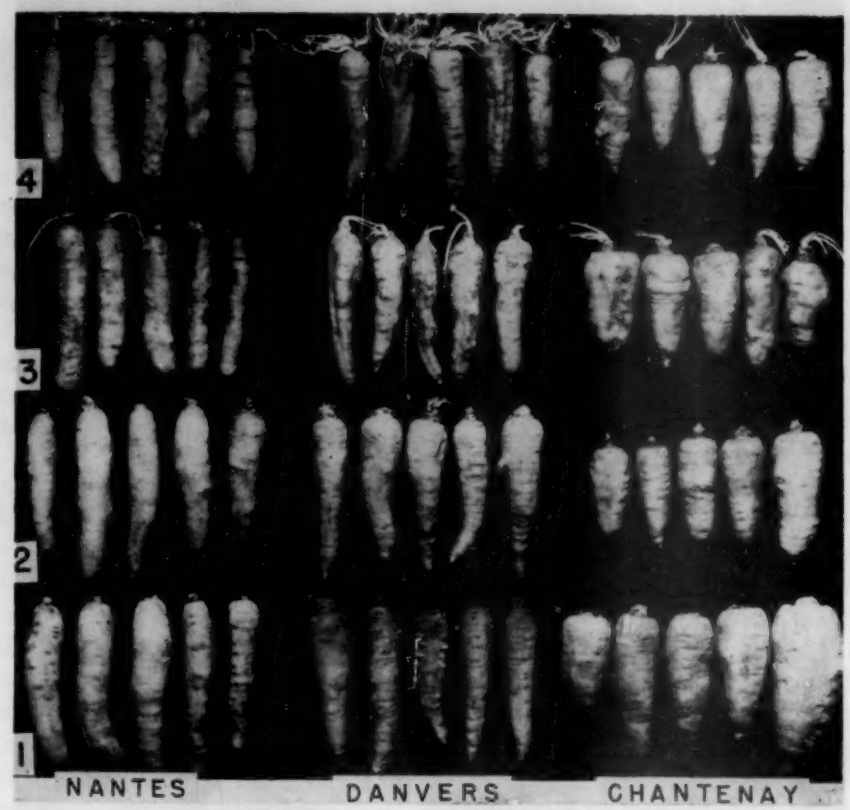

FIG. 2. The sprout inhibiting effects of preharvest foliage sprays of growth regulators on Nantes, Danvers, and Chantenay carrots: (1) benzo-thiazol-2-oxyacetic acid, 2500 p.p.m., (2) maleic hydrazide, 2500 p.p.m. (3) sodium salt of alpha-naphthaleneacetic acid, 5000 p.p.m., and (4) non-treated controls.

The other treatments caused no significant inhibition of sprouting in onions. As indicated in table III, the sodium salt of alpha-naphthaleneacetic acid and 2,4,5-trichlorophenoxyacetic acid resulted in a significantly greater weight of sprouts, and the "Barsprout" formulation significantly increased the percentage of rotten bulbs. With the exception of the maleic hydrazide treatments, the "blowout" defect was common on all bulbs subjected to the various chemical treatments. This defect was particularly associated with chemicals stimulating sprouting and the usual accompanying storage decay and breakdown.

Discussion

Chemical inhibition of sprouting and root growth on stored onions and carrots by preharvest foliage sprays offers an interesting field for further study. The convenience of applying the substance to plants in the field

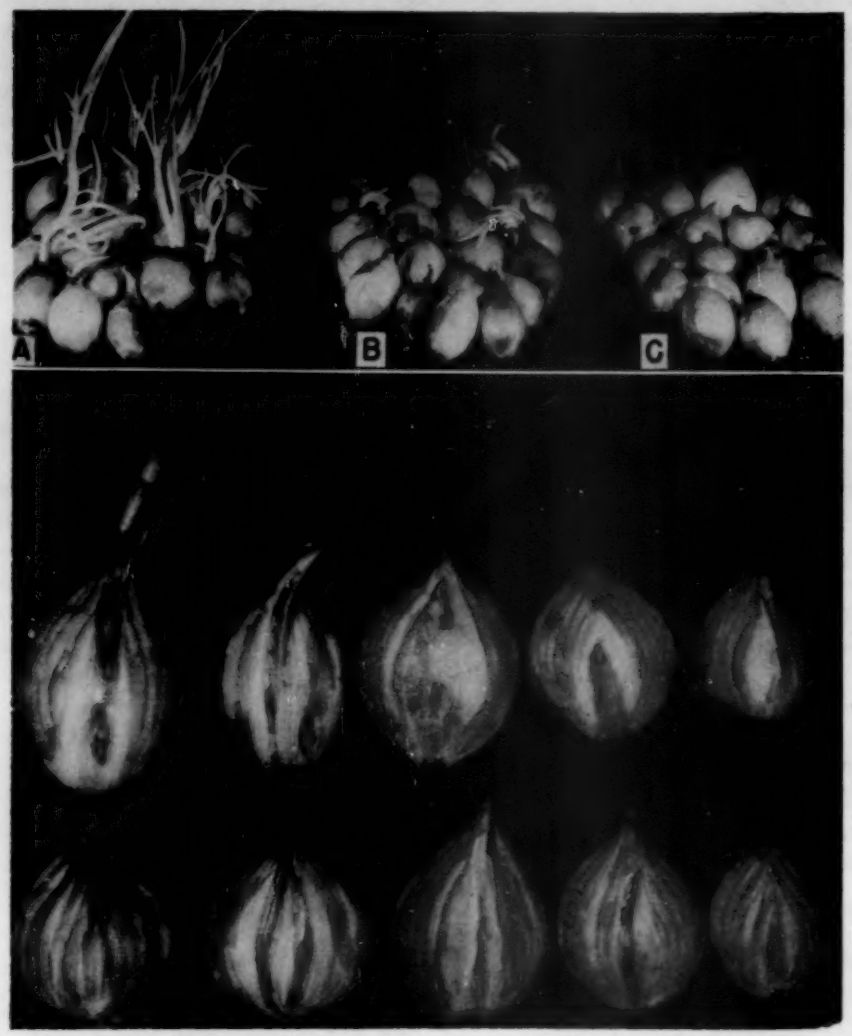

FIG. 3. The effects of preharvest foliage sprays of maleic hydrazide on sprouting of Sweet Spanish onions: Bottom, longitudinal sections of bulbs harvested from plants which received 2500 p.p.m. of maleic hydrazide, and above, longitudinal sections of non-treated controls; top (A), controls, (B), maleic hydrazide, 500 p.p.m., and (C), maleic hydrazide, 2500 p.p.m.

would eliminate many of the present difficulties incurred by directly treating the bulbs or roots. Preharvest foliage spray treatments also have the added advantage in that the food product is treated only indirectly. The

fact that subsequent storage sprouting and rooting can be controlled by foliar field treatments suggests that the growth regulators themselves or some associated effects are translocated downward to the growing points and perhaps concentrated in tissues having the greatest metabolic activity. Thus, the actual amount of chemical in the edible product is likely to be lower than that necessary for similar growth inhibition where it is applied directly to the harvested produce.

The various treatments had no effect on yield. The chemicals were applied on onions two weeks and on carrots four days prior to harvesting. In both instances the crops had reached acceptable maturity before being treated.

On carrots the benzo-thiazol-2-oxyacetic acid resulted in almost complete growth inhibition when used at 2500 p.p.m. but produced no measurable retardation of sprouting on onions. The effect of the material was unusual considering its very marked inhibition of sprout growth in carrots. There was less of the accompanying stimulation of callus tissue and rooting in the areas where the lateral roots had appeared. However, in onions benzo-thiazol-2-oxyacetic acid stimulated the "blowout" condition to develop characteristic of the sodium salt of alpha-naphthaleneacetic acid, "Barsprout," and 2,4,5-trichlorophenoxyacetic acid. Benzo-thiazol-2-oxyacetic acid deserves further investigation as a possible plant growth inhibitor.

The most striking and consistent results on sprout inhibition with both carrots and onions were obtained with maleic hydrazide. Furthermore, the inhibitions of sprouting induced by maleic hydrazide were not accompanied by the growth irregularities peculiar to the other growth substances. There were no significant alterations of major nutrient constituents associated with the induced dormancy in carrots. First described by SCHOENE and HOFFMAN (**6**) as a unique growth regulant, maleic hydrazide has since been reported as having many unusual effects on plant growth and development (**3, 5, 6, 11, 12**). Retardations of sprout and root growth induced by maleic hydrazide on carrots and onions apparently represent true growth inhibitions and are not accompanied by concurrent stimulations of abnormal growths so characteristic of the other chemicals used. The storage life of Sweet Spanish onions was prolonged not only by an absence of sprout growth but by a reduction in breakdown of bulbs frequently associated with sprout production.

Summary

The effects of preharvest foliage sprays of several growth regulators on sprout inhibition and storage quality of Danvers, Nantes, and Chantenay carrots and yellow Sweet Spanish onions were determined.

Sprouting and root growth of onions held in a 55° F storage for five months were completely inhibited by the application of a water spray containing 2500 p.p.m. of maleic hydrazide to the foliage of the intact plants two weeks before harvesting the bulbs. Partial sprout inhibition was

achieved by 500 p.p.m. of maleic hydrazide. Other chemicals either gave no significant control of sprouting or stimulated it, and storage breakdown was in some instances more prevalent than in controls.

Sprout growth of carrots held at 50° F for three months was almost completely inhibited by foliar sprays of 2500 p.p.m. of both maleic hydrazide and benzo-thiazol-2-oxyacetic acid applied four days before harvesting. The maleic hydrazide produced no significant alteration in the amount of dry matter, carotene, carbohydrates or Kjeldahl nitrogen in the stored carrots. Sprouting in carrots was also significantly reduced by foliar sprays of the sodium salt of alpha-naphthaleneacetic acid, 1000 and 5000 p.p.m. and by 500 p.p.m. of either maleic hydrazide or benzo-thiazol-2-oxyacetic acid.

Accompanying the use of chemicals other than maleic hydrazide and to a lesser extent benzo-thiazol-2-oxyacetic acid on carrots were frequent undesirable formations of callus tissues, proliferations and other abnormalities which tended to shorten rather than prolong the storage life of both carrots and onions.

The authors wish to acknowledge the help of the analytical group in the Department of Agricultural Chemistry with some of the chemical analyses.

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INTERACTION OF CYSTINE AND CASEIN HYDROLYSATE DURING THE AUTOCLAVING OF A MICROBIOLOGICAL ASSAY MEDIUM

R. F. Dawson and Hope H. Robson

Received May 29, 1950

Introduction

Rose and Peterson (**6**) have reported that the rates of acid production by *Lactobacillus arabinosus* (ATCC 8014), *L. casei* (ATCC 7569), and *Streptococcus faecalis* (ATCC 9790) were less where the remainder of the ingredients of the culture medium were autoclaved in the absence of the dextrose component. Rabinowitz and Snell (**5**) had already found that autoclaving was necessary to support growth of *S. faecalis* on their medium. However, overautoclaving rendered the medium incapable of supporting maximum growth due to the destruction of cystine and cysteine. It is thus generally agreed (**6**) that the effects of heat upon bacteriological culture media containing a reducing sugar, while complex, are not always entirely deleterious.

In the course of attempts to eliminate "drift" in assay values for niacin using *Lactobacillus arabinosus* (ATCC 8014) as a test organism we have found that separate autoclaving of dextrose and the remaining culture medium components leads to an operationally induced cystine deficiency for this organism. The present paper reports upon the nature of such loss and upon its counteraction by dextrose.

Experimental

In these experiments, the procedures of the Association of Vitamin Chemists (**3**) have been followed with certain exceptions. Potassium acetate was substituted for sodium acetate, and the concentration was increased to 1.5%. Glucose was supplied at a concentration of 1.2%. The pH of the basal medium was reduced to 6.4 after extensive preliminary experimentation had shown this to be an optimum value for media destined to be autoclaved. The test cultures were incubated at 34° C for 66–72 hours. Acid production was determined by titration with 0.1 N NaOH at the glass electrode. Sterilization of all solutions by autoclaving was accomplished by heating at 15 lbs. (steam) for 15 minutes. In some cases solutions were sterilized by filtration through Pyrex sintered glass funnels.

Each experimental treatment involved two complete sets of niacin dilutions covering the usual range, 0–0.40 microgram of niacin per tube, in increments of 0.05 microgram. The titration values for such duplicated sets were compared in detail with those from a series of standard curves relating niacin dosage to acid production under standardized conditions. For the purpose of the present communication, an index of behavior was

obtained by subtracting the mean titration value at the 0.30 microgram level of niacin dosage from the corresponding mean titration value for the standards after each had been corrected for its respective blank titration. The mean titration value at the 0.30 microgram level of niacin dosage for 15 standard curves was 9.4 ml. of 0.1 N NaOH with a standard deviation of 0.37 ml. Deviations from this mean value of less than 0.7 ml. were regarded as insignificant and listed as 0 in the tables.

Results and discussion

When cystine was omitted from the basal medium, acid production was reduced more than 50% (table I). It is thus evident that a requirement

TABLE I

DECREASE IN ACID PRODUCTION BY *Lactobacillus arabinosus*

TREATMENT	*DECREASE IN TITRATION
	†ml. per tube
Glucose autoclaved separately	3.4, 4.8, 5.1
Glucose filter sterilized separately	4.0
Glucose autoclaved separately with 1 mg. tryptophan added	3.5
Glucose autoclaved separately with 2 mg. methionine added	3.7
Glucose autoclaved separately with 1.5 mg. glutamic acid added	4.1
Glucose autoclaved separately with 1 mg. cystine added	1.1
Glucose autoclaved separately with cystine and vitamin B_6 added	1.8
Glucose autoclaved separately with cystine, vitamin B_6 and phosphate	1.7
Glucose autoclaved separately with 3–6 mg. cysteine added afterward	0, 0
Cystine omitted from basal medium	5.1
Casein autoclaved separately	0
Casein autoclaved with tryptophan	0
Casein autoclaved with buffer	0
Casein autoclaved with buffer and tryptophan	0
Cystine autoclaved separately	0
Cystine autoclaved with casein	1.7, 2.6
Cystine autoclaved with casein and tryptophan	2.5
Cystine autoclaved with casein and buffer	3.3
Cystine autoclaved with casein, buffer and tryptophan	3.5, 4.0
Cystine autoclaved with casein, buffer, tryptophan and glucose	0

All figures represent net reduction in titratable acidity occasioned by the treatments listed. Each treatment was applied to duplicated series of cultures covering the usual range of niacin dosage, 0–0.4 microgram per tube, but for convenience only the data at the 0.30 microgram dosage are given in the table.

* The basis for comparison was the mean titration value at the 0.30 microgram level of niacin dosage for standard curves obtained in the usual way and presumably representing approximately optimum rates of acid production.

† 0.1 N NaOH.

for cystine existed under the conditions employed in these experiments. Consequently, it is of interest to note that the effect upon acid production of autoclaving culture medium in the absence of glucose (table I) was quantitatively of the same order of magnitude. Indeed, it was immaterial whether the glucose was sterilized by autoclaving or by filtration. Repeated experiments in which glucose has been autoclaved with various amounts of

acetate buffer and at various pH levels have failed to show any important effect of breakdown products of glucose as such upon the ability of the bacteria to produce acid.

Likewise, autoclaving glucose with extra tryptophan, methionine, or glutamic acid in 1% potassium acetate did not markedly alter the extent of the decrease in acid production. It would hence seem, in agreement with the findings of ROSE and PETERSON (**6**), that the products of the so-called Maillard reaction were not responsible for the inhibition.

The first observation relating cystine to the source of the inhibitions in acid production noted above came from combining extra cystine with glucose during separate autoclaving of the latter. When 1 mg. of cystine, 0.10 g. of glucose and 12 mg. of potassium acetate per ml. of water were separately autoclaved and then added aseptically in these amounts to 9 ml. of the autoclaved remainder of the culture medium, the decrease in acid production was much less (table I). The addition of pyridoxine alone or of pyridoxine and extra salts A (containing phosphate) to the combination of cystine, buffer and sugar before autoclaving did not significantly alter the magnitude of this response.

The second observation of importance is that the addition of filter-sterilized cysteine to the ingredients following their separate heat sterilization entirely removed the inhibition of acid production.

Filter sterilization of the whole medium gave maximum acid production; and the common denominator in the foregoing experiments, therefore, would seem to be the effect of heat (autoclaving) upon the amino acid-vitamin-salt component of the culture medium in the absence of glucose. Whatever the mechanism of this interaction, it would appear to involve the development of limiting concentrations of cystine. Further experiments have thrown some light upon the nature of this interaction.

Cystine was withheld during the preparation of the basal medium and autoclaved separately under a variety of circumstances. Separate autoclaving of cystine alone and of the remainder of the basal medium minus cystine did not yield a depressing effect upon acid production when these components were subsequently combined under aseptic conditions, inoculated and cultured in the usual way (table I). Systematic withdrawal and separate autoclaving of various other ingredients of the basal medium gave the following results. Autoclaving cystine with casein gave a depressing effect of 2 ml. of 0.1 N alkali or more, and this effect was not altered by the inclusion of tryptophan. Addition of potassium acetate buffer to cystine and casein hydrolysate still further depressed acid production, and the effect was again not appreciably altered by the inclusion of tryptophan. Separate autoclaving of the casein component of the basal medium alone or in combination with tryptophan and buffer did not depress acid production.

The conclusion appears to be that during autoclaving, cystine interacts with some component or components of the casein hydrolysate in such a manner that the concentration of cystine in the culture medium is reduced

to a limiting level. This interaction appears to be facilitated by acetate buffer. Tryptophan is not involved nor is vitamin B_6. The interaction is overcome by the inclusion of glucose prior to autoclaving, by addition of extra cystine to the separately autoclaved glucose, or by addition of cysteine after autoclaving the whole medium in the culture tubes.

The nature of the interaction is not clearly revealed by these experiments. From the evidence available it seems possible that heat promotes a reaction between the zwitterion form of cystine and some other amino acid(s) the products of which (such as diketopiperazines, for instance) are only slowly available to the organism for supplying the cystine requirement. The manner in which glucose prevents the heat inactivation of cystine is likewise obscure, but in view of the fact that sucrose cannot entirely replace glucose as a substrate for acid production by this organism, it would seem that the reducing group of glucose may be a factor in the protective action. Simple model experiments can be devised to show that cystine is reduced to cysteine by the ferrous iron of the salts B during the preparation of the basal medium. It is possible that cysteine so formed may escape piperazine formation by reacting with glucose in the manner proposed by AGREN (**1, 2**) in his papers on the thermal reactions of these two substances. One need then only postulate the ready availability to Lactobacillus of Agren's thiazole derivative of cysteine and glucose in order to explain the results contained herein. Repeated attempts have been made in this laboratory to isolate a strain of *L. arabinosus* which can use sucrose as readily as glucose. In spite of these efforts and of the excellent results obtained by HILL and PATTON (4) with *Streptococcus faecalis*, we have been unable to secure more than 80–90% of maximum acid production by using sucrose alone. That the relative ineffectiveness of sucrose cannot be due entirely to a slower rate of utilization was shown by replacing one tenth of the sucrose content of the basal medium with glucose. By this means acid production from sucrose was raised to a level equal to that from an equivalent concentration of glucose. It would thus seem that some small amount of glucose is needed in the basal medium when the latter is to be sterilized by autoclaving in order to prevent the development of cystine deficiency with consequent limitations to the rate of acid production.

Summary

The depressing effect of autoclaving glucose separately from the remaining components of the culture medium upon acid production by *Lactobacillus arabinosus* has been investigated. This effect was quantitatively similar to that obtained by withholding cystine from the medium.

The effect could be largely or wholly removed by adding glucose to the medium before autoclaving; by autoclaving glucose in the presence of additional cystine; and by adding cysteine after separate autoclaving.

The effect was traced to an interaction between cystine, casein hydroly-

sate and heat. The interaction was enhanced by the presence of acetate buffer.

The effect could be obtained, in reduced degree, by replacing glucose with sucrose prior to autoclaving the whole medium. Since one-tenth of the sucrose could be replaced with glucose to remove the inhibitory effect completely, it is suggested that the reducing group of glucose is involved in the protective action of this sugar on cystine.

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MOLYBDENUM NUTRITION OF ALFALFA[1]

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(WITH TWO FIGURES)

Received June 15, 1950

Molybdenum is the latest element to be found essential for microörganisms and higher plants. The literature, recently reviewed by Mulder (**12**), indicates that Mo is necessary for N fixation by Azotobacter and Rhizobium and for the metabolism of nitrates by all plants. The biological importance of Mo was originally discovered by Bortels (**3, 4**), who reported that Azotobacter and peas, soybeans, and red clover showed increases in growth and in N fixation from additions of Mo. Essentiality of Mo for higher plants was first demonstrated by Arnon and Stout in 1939. Using highly purified nutrient solutions, they produced Mo-deficiency symptoms in tomatoes in six successive experiments. Later Jensen and Betty (**9**) and Jensen (**10**) reported that small applications of Mo to alfalfa and clover growing in pots of sand increased the yields of tops and the N content of both tops and roots. Mulder showed that nodulated peas grown in culture solutions lacking both Mo and combined N quickly developed N-deficiency symptoms. The Mo requirement of tomatoes was greatly reduced when ammonium N was substituted for nitrate N in the nutrient solutions. Recent work by Hewitt and Jones (**8**) indicates that Mo is indispensable for the growth of tomatoes, cauliflower, cabbage, and mustard and that it functions in the reduction of nitrates. Vanselow and Datta (**15**), using the spectrographic method, studied the Mo requirements of lemons and found that leaves containing 0.01 p.p.m. Mo were deficient but those containing concentrations greater than 0.024 p.p.m. were normal. Deficiency was produced with either nitrate or ammonia N.

Further investigation of the importance of Mo in plant nutrition has been stimulated by reports of Mo-deficient soils in Australia (**1, 2**), California (**15**) and, more recently, in New Jersey (**5, 7**). In studying the Mo status of New Jersey crops and soils, an effort was made to obtain additional information on Mo-deficiency symptoms, the quantities of Mo required, and the effects of Mo additions on the Mo and N content of the plants. Special purification and cultural techniques were developed for studying the Mo nutrition of alfalfa.

Purification and analytical methods

Although Mo is not ordinarily added to nutrient solutions prepared from distilled water and analytical grade chemicals, normal plant growth usually results. The plants obtain sufficient Mo from the impurities in

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Department of Soils, New Brunswick, New Jersey.

these solutions or from the containers. Analyses of the greenhouse supply of distilled water showed a Mo content of 0.08 to 0.1 part per billion. Samples of C.P. Fe salts contained up to 0.1 p.p.m. Mo. These impurities had to be removed before the Mo requirements of plants could be studied.

The necessary water for the preparation of nutrient solutions was obtained by redistilling the regular supply of distilled water in an automatic pyrex glass still designed in this laboratory. Pyrex glassware was used throughout the experiment. It was cleaned with hot concentrated HCl and then rinsed with redistilled water. All solutions were stored in pyrex-glass bottles that were covered with Petri dishes or beakers.

The basic nutrient solution consisted of 0.005 M $Ca(NO_3)$ or $CaCl_2$, 0.0005 M KH_2PO_4, 0.002 M $MgSO_4$, 0.0025 M K_2SO_4, 0.25 p.p.m. B as $Na_2B_4O_7$, 0.25 p.p.m. Mn as $MnSO_4$, 0.25 p.p.m. Zn as $ZnSO_4$, 0.5 p.p.m. Fe as a mixture of $FeCl_2$ and $FeCl_3$, 0.1 p.p.m. Cu as $CuSO_4$, and 0.015 p.p.m. Mo as Na_2MoO_4. The solution had a pH value of 5.1. With the exception of $CaCl_2$, the nutrient salts were purified by recrystallizing C.P. salts twice from redistilled water. The recrystallization was accomplished by saturating boiling water with the salt, and then filtering and cooling. Yields were enhanced in some cases by adding glass-distilled ethanol. Since $CaCl_2$ is difficult to recrystallize, it was prepared by neutralizing freshly precipitated $CaCO_3$, made from Na_2CO_3 and $CaCl_2$ solutions, with glass-distilled HCl. Stock solutions, 0.5 M in concentration, were prepared from all the macro-nutrient salts, each of which was further purified by the 8-hydroxyquinoline coprecipitation process described by Hewitt and Jones (**8**). Soluble quinolates left in the filtrate were extracted with redistilled chloroform. With the exception of Fe, stock solutions of all micronutrients were prepared and used without additional purification.

Since Fe salts were known to contain Mo, they were further purified by the following method:

A solution containing 1500 p.p.m. Fe was prepared from recrystallized $FeSO_4$ and placed in a two-liter separatory funnel. The acid concentration was adjusted to 5% with redistilled HCl, then 0.6 gm. KSCN was added for each 100 ml. of solution. Saturated Na_2S solution was added as a reducing agent until the color due to $Fe(SCN)_3$ disappeared. The Mo-thiocyanate complex was removed by three extractions with redistilled isopropyl ether. The aqueous solution of Fe was warmed and filtered and $Fe(OH)_2$ was precipitated with redistilled NH_4OH. The $Fe(OH)_2$ was washed free of KSCN and neutralized with redistilled 1-N HCl until the pH value was between 2 and 3. The concentration of Fe in the resulting mixture of $FeCl_2$ and $FeCl_3$ was determined by analysis, and a stock solution containing 1000 p.p.m. Fe was prepared by dilution. Titration with $KMnO_4$ showed that 76% of the total Fe was in the divalent form.

After the alfalfa had been grown to the early bloom stage, the tissues were harvested and oven-dried at 70° C, ground in a Wiley mill (check analyses showed no contaminating effect), and analyzed for Mo by a modi-

fication (**6**) of the thiocyanate stannous chloride method. Total N was determined by the Kjeldahl method modified to include nitrates. Nitrate N was determined by the method described by SNELL and SNELL (**12**).

Cultural methods

Four-liter pyrex glass beakers, fitted with one-fourth-inch drain holes, were used as culture vessels. These were filled with acid- and alkali-washed pyrex glass cullet crushed to pass a one-fourth-inch screen. Twenty-five gm. of acid-washed pyrex glass wool was mixed with the cullet in each beaker to increase the water-holding capacity. The crushed glass was leached with 1-N redistilled HCl and with redistilled water until the pH value of the effluent was above five. The culture vessels were placed inside two-gallon ceramic jars fitted with "Vinylite" covers (fig. 1). The

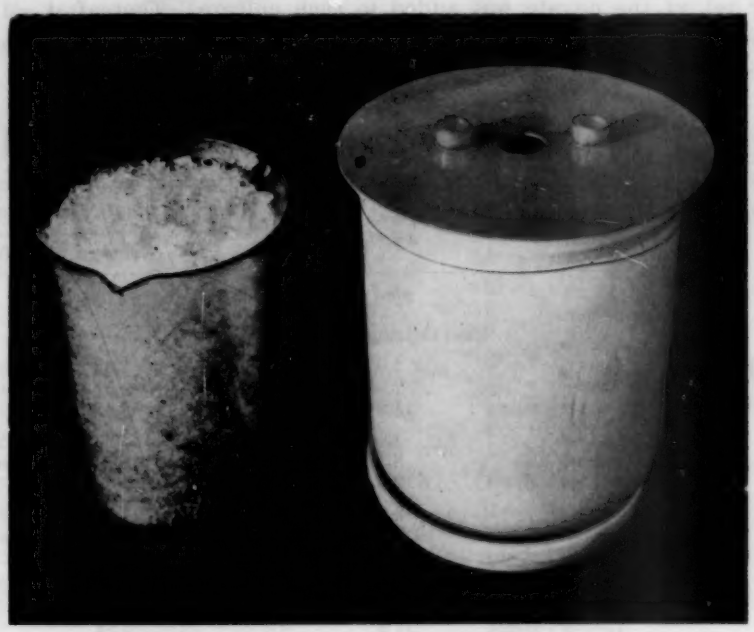

FIG. 1. Culture vessels for study of Mo requirements of alfalfa. The beaker, filled with crushed pyrex glass, was placed inside the ceramic jar.

neck of a 50-ml. Erlenmeyer flask was placed in the center opening of each cover. Each flask had four small holes in its bottom through which the nutrient solutions were dispersed. The flasks were kept covered at all times, except when solutions were being added.

Seedlings of Atlantic alfalfa were grown in pyrex dishes of acid-washed pyrex glass sand and supplied with one-half strength purified nutrient solution without Mo. On February 10, 1949, uniform seedlings approximately four inches in height were transferred to twelve culture vessels. Two plants were supported in each vessel by glass wool fitted in the openings of

the plastic covers. Cultures were divided into four series of triplicate pots and distributed at random on a bench in the greenhouse. Each culture was supplied with one liter a day of purified nutrient solution.

In the first three cuttings, one series was supplied with nutrient solution containing nitrate N and 0.015 p.p.m. Mo. The other three series received identical solutions with the exception of Mo. In the fourth cutting all plants received nitrate N and Mo at rates outlined in table I. After the fourth harvest, all cultures were flushed five times with redistilled water to remove nitrates and the plants were inoculated with Rhizobium by the following method: Two nodule masses from an alfalfa plant in the field were washed with redistilled water and macerated in a mortar. Six hundred ml. of purified nutrient solution, without nitrates, was added to the macerated nodules and the solution was filtered through coarse filter paper. Fifty ml. of the filtrate was added to each culture. Thereafter all cultures were supplied with nutrient solutions containing $CaCl_2$ instead of

TABLE I

EFFECT OF Mo ON YIELD,* AND ON Mo AND N CONTENT OF ALFALFA SUPPLIED WITH NITRATE N

Mo treatment	Plant portion**	Dry wt.†	Mo††	N content	
				Nitrate	Total
		gm.	*p.p.m.*	*mg./gm.*	*%*
		First Cutting—April 27			
0.015 p.p.m.	Leaves	3.9	1.5	2.62	3.68
	Stems	7.9	1.2	2.31	2.87
	Leaves + stems	11.8	1.3	2.42	3.22
None	Leaves	4.4	0.0	4.65	2.89
	Stems	8.1	0.0	2.79	2.32
	Leaves + stems	12.5	0.0	3.54	2.52
None	Leaves	3.8	0.0	3.32	3.22
	Stems	7.1	0.0	2.61	2.29
	Leaves + stems	10.9	0.0	2.86	2.62
None	Leaves	4.2	0.0	3.38	3.16
	Stems	7.1	0.0	3.17	2.69
	Leaves + stems	11.3	0.0	3.24	2.86
		Second Cutting—June 3			
0.015 p.p.m.	Leaves	8.9	5.3	2.22	4.05
	Stems	17.6	3.3	1.85	2.47
	Leaves + stems	26.5	4.0	1.97	3.01
None	Leaves	6.4	0.0	2.91	3.42
	Stems	12.9	0.0	2.43	2.01
	Leaves + stems	19.3	0.0	2.58	2.48
None	Leaves	5.6	0.0	2.98	3.47
	Stems	11.3	0.0	2.66	1.81
	Leaves + stems	16.9	0.0	2.76	2.36
None	Leaves	5.3	0.0	3.01	3.36
	Stems	13.1	0.0	2.88	1.62
	Leaves + stems	18.4	0.0	2.92	2.12

TABLE I (*Con't*)

Mo treatment	Plant portion**	Dry wt.†	Mo††	N content	
				Nitrate	Total
		gm.	*p.p.m.*	*mg./gm.*	%
		Third Cutting—July 5			
0.015 p.p.m.	Leaves	9.6	8.9	2.40	3.94
	Stems	10.6	4.9	2.05	1.68
	Leaves + stems	20.2	6.8	2.22	2.75
None	Leaves	6.6	0.0	2.81	3.30
	Stems	6.7	0.0	2.66	1.40
	Leaves + stems	13.3	0.0	2.73	2.35
None	Leaves	6.7	0.0	2.82	3.44
	Stems	7.3	0.0	2.41	1.71
	Leaves + stems	14.0	0.0	2.61	2.54
None	Leaves	6.1	0.0	2.94	3.36
	Stems	6.9	0.0	2.72	1.76
	Leaves + stems	13.0	0.0	2.82	2.51
		Fourth Cutting—July 27			
0.015 p.p.m.	Leaves	3.7	7.4	2.49	4.50
	Stems	4.1	4.2	2.00	2.04
	Leaves + stems	7.8	5.7	2.23	3.22
0.10 p.p.m.	Leaves	2.8	9.8	2.52	4.04
	Stems	3.6	5.6	2.18	1.71
	Leaves + stems	6.4	7.4	2.33	2.73
None	Leaves	2.6	0.0	3.46	3.75
	Stems	3.1	0.0	2.65	1.68
	Leaves + stems	5.7	0.0	3.01	2.62
0.001 p.p.m.	Leaves	3.0	0.2	2.41	4.52
	Stems	3.5	0.1	2.25	1.76
	Leaves + stems	6.5	0.1	2.32	3.03

* First cutting: no significant difference in yield.
Second cutting: increase in yield of leaves + stems from Mo treatment significant at 2% level.
Third cutting: increase in yield of leaves + stems from Mo treatment significant at 1% level.
Fourth cutting: L.S.D. in yield of leaves + stems at 1% level = 1.8 gm., at 5% level = 0.8 gm.
** Petioles are included with leaves.
† Mean yield of three cultures.
†† 0.0 = less than 0.1 p.p.m. Mo.

$Ca(NO_3)_2$ and Mo at rates given in table II. The fifth and sixth cuttings were grown on atmospheric N.

Responses when supplied with nitrates

No yield responses were obtained from Mo additions in the first cutting (table I) but the untreated plants exhibited deficiency symptoms. They were generally light green and their lower leaves were chlorotic. After the first cutting was harvested, Mo-treated plants grew more rapidly, were darker green, and appeared more vigorous than those receiving no Mo. At the time of the second harvest, plants without Mo had devel-

oped severe chlorosis and a scorched appearance of the lower leaves. Responses to Mo were significant at the 2% level in this cutting. In the third cutting, plants not receiving Mo showed the same deficiency symptoms, and a highly significant increase in yield from Mo was obtained.

After the third harvest, Mo was applied at two additional rates. The fourth cutting was harvested 22 days later, since the intense daytime heat in the greenhouse caused wilting of the leaves. The plants without Mo showed the typical deficiency symptoms, but the treated plants were normal. Additions of Mo at rates of 0.015, 0.10, and 0.001 p.p.m. increased yields 37, 12, and 14%, respectively, over the untreated series. The yield increase for the 0.015 treatment is not comparable with those for the 0.10 and 0.001 Mo treatments, since the former received Mo for the first three cuttings.

The untreated plants contained less than 0.1 p.p.m. Mo in all cases. Identically treated plants harvested early in spring contained less Mo than those that were harvested in June or July. This difference was probably caused by variable light conditions or by an accumulation of Mo in the culture media. The Mo concentration in plants from the fourth cutting showed a close correlation with the quantities of Mo applied. Plants supplied with solutions containing 0.001 p.p.m. Mo contained a mean of 0.1 p.p.m. Mo. Leaves from treated plants consistently contained over 50% more Mo than the stems.

Molybdenum had a pronounced effect on the N content of both leaves and stems. The total N in Mo-treated plants from the first cutting ranged between 13 and 28% higher than that of the untreated plants. This relationship was consistent in all cuttings. In the second cutting, plants receiving Mo contained 13 to 28% more total N than untreated plants, and 8 to 17% more in the third. Additions of Mo at rates of 0.015, 0.1, and 0.001 p.p.m. in the fourth cutting increased the total N concentration of the plants by 23, 4, and 16%, respectively. Plants grown without Mo accumulated nitrates in their tissues, but not to such an extent as has been reported for non-legumes (**8**).

Responses when grown on atmospheric nitrogen

After the fourth cutting was harvested, the cultures were removed from the jars and the roots were examined for nodules. Many small roots were concentrated on the bottoms and along the sides of the beakers, but no nodules were observed. The beakers were replaced in the jars, flushed with water, and inoculated as previously described. The fifth and sixth cuttings were grown without combined N.

All plants grew very slowly for three weeks after the inoculation and developed typical N-deficiency symptoms. The upper leaves were light green and many of the lower leaves became necrotic and abscissed. These symptoms increased in severity until September 23, when plants receiving Mo showed some evidence of recovery. Plants treated with Mo at rates of

0.015, 0.10, and 0.001 p.p.m. rapidly regained their normal color and succulence, but those without Mo remained chlorotic. By August 24, necrosis had developed on the margins of many of the upper leaves of untreated plants. The symptoms differed from those of N-deficiency. The third cutting was harvested on August 30. The series receiving Mo at rates of 0.015, 0.10, and 0.001 p.p.m. yielded 71, 63, and 63% more dry matter, respectively, than the untreated series (table II). These responses were highly significant.

TABLE II

EFFECT OF APPLICATIONS OF Mo ON YIELD* AND ON Mo AND N CONTENT OF ALFALFA GROWN ON ATMOSPHERIC N

Mo treatment	Plant portion**	Dry wt.†	Mo††	Total N
		gm.	*p.p.m.*	*%*
	Fifth Cutting—August 30			
0.015 p.p.m.	Leaves	3.7	6.7	3.75
	Stems	3.3	3.1	1.65
	Leaves + stems	7.0	5.0	2.77
0.10 p.p.m.	Leaves	3.6	13.2	4.36
	Stems	3.1	6.3	1.57
	Leaves + stems	6.7	10.0	3.07
None	Leaves	2.1	0.0	2.54
	Stems	2.0	0.0	1.20
	Leaves + stems	4.1	0.0	1.88
0.001 p.p.m.	Leaves	3.6	0.6	4.06
	Stems	3.1	0.3	2.24
	Leaves + stems	6.7	0.5	3.21
L. S. D. in yield of leaves + stems at 1% level = 1.2 gm., at 5% level = 0.8 gm.				
	Sixth Cutting—October 14			
0.015 p.p.m.	Leaves	9.0	6.2	4.03
	Stems	6.6	3.5	2.26
	Leaves + stems	15.6	5.1	3.28
0.10 p.p.m.	Leaves	9.8	11.3	4.17
	Stems	6.3	6.7	2.10
	Leaves + stems	16.1	9.5	3.36
None	Leaves	2.0	0.0	2.24
	Stems	1.6	0.0	1.15
	Leaves + stems	3.6	0.0	1.76
1 microgram§	Leaves	3.9	0.0	3.42
	Stems	3.8	0.0	1.90
	Leaves + stems	7.7	0.0	2.67
0.001 p.p.m.	Leaves	10.3	0.3	4.06
	Stems	6.0	0.1	2.18
	Leaves + stems	16.3	0.2	3.36

* Fifth cutting: L. S. D. in yield of leaves + stems at 1% level = 1.2 gm., at 5% level = 0.8 gm.

Sixth cutting: L. S. D. in yield of leaves + stems at 1% level = 2.2 gm., at 5% level = 1.2 gm.

** Petioles included with leaf portion.

† Mean yield for three cultures, except the no Mo and 1 microgram treatment in the sixth cutting.

†† 0.0 = less than 0.1 p.p.m. Mo.

§ One microgram Mo added after fifth harvest.

Immediately after the fifth harvest, one deficient culture was treated with one microgram Mo and, thereafter, it was flushed with a purified nutrient solution containing no Mo. For two weeks after treatment, the plants in this culture grew normally, with no evidence of deficiency symptoms. During the same period the plants in untreated cultures developed a general chlorosis of their upper leaves and a pronounced necrosis of many lower leaves (fig. 2). On October 14, the deficient plants showed necrosis

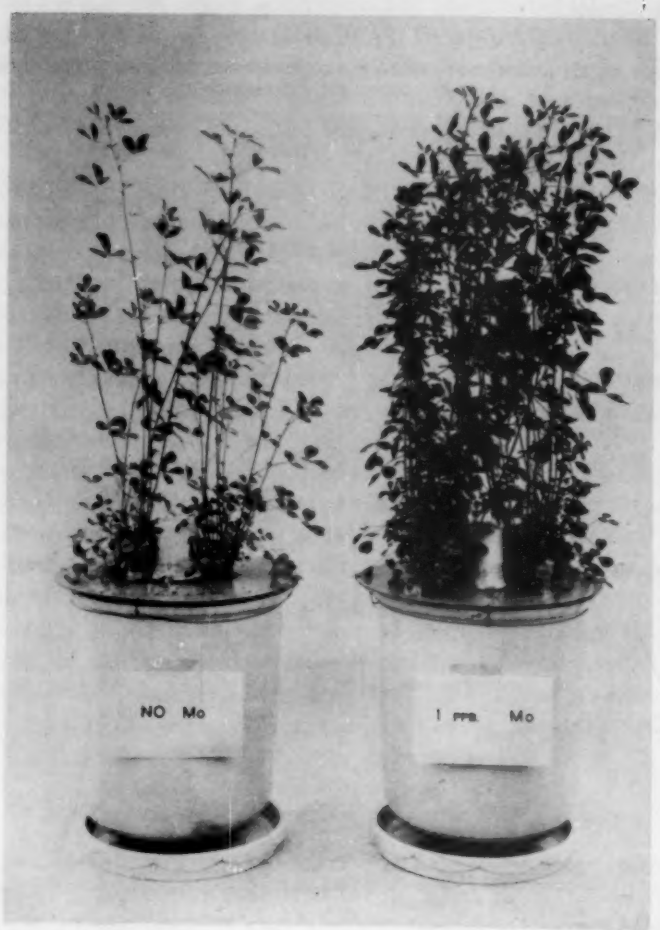

FIG. 2. Effect of Mo on growth of Atlantic alfalfa utilizing atmospheric N. Left, no Mo; right, 1 part Mo per billion of solution.

of the tips and margins of many upper leaves, but no deficiency symptoms were apparent in any of the Mo-treated plants. This was considered conclusive evidence that the symptoms observed in untreated cultures were caused by Mo deficiency and not by lack of inoculation.

In the sixth cutting, plants receiving Mo at rates of 0.015, 0.10, and 0.001 p.p.m. yielded between 334 and 352% more dry matter than the un-

treated plants. The plants that were treated with only one microgram Mo produced 114% more dry weight than those receiving no Mo. Under the conditions of this experiment, 0.001 p.p.m. Mo in nutrient solutions was required for alfalfa grown on atmospheric N and was sufficient for maximum yield.

The analytical data for the fifth and sixth cuttings show the same correlation between quantities of Mo added and the Mo content of the plants as was observed in the first four cuttings. Deficient plants in both cuttings contained less than 0.1 p.p.m. Mo in either stems or leaves. The plants treated with Mo at the rate of 0.001 p.p.m. had a mean Mo content of 0.5 p.p.m. in the fifth cutting and of 0.2 p.p.m. in the sixth. These concentrations in tissues were sufficient for maximum yield and N fixation. No Mo was detected in plants from cultures receiving one microgram Mo, yet they showed marked response to this small addition.

All Mo-treated plants in both the fifth and sixth cuttings contained more total N than the untreated plants. The increases for the 0.015, 0.10, and 0.001 p.p.m. Mo applications were 47, 63, and 71%, respectively, in the fifth cutting, and 86, 91, and 92% in the sixth. Plants treated with one microgram Mo contained 52% more total N than those untreated plants. These data provide conclusive evidence of the importance of Mo for N fixation by alfalfa.

Yields and analyses of roots

The roots were removed from the pots on December 2, washed, and examined. Most of the nodules were found on the upper large roots and very few on the fine roots, which were concentrated in the bottoms of the beakers. The data in tables II and III show no correlation between numbers of nodule masses and either yield or N content of roots, stems, and leaves. Since a large number of nodules were found on Mo-deficient roots, it is concluded that the N-fixing bacteria were parasitic on these roots. Roots of plants that had been supplied with Mo produced greater dry weights

TABLE III

EFFECT OF APPLICATIONS OF MO ON YIELD, NUMBER OF NODULES, AND MO AND N CONTENT OF ALFALFA ROOTS

HARVESTED DECEMBER 2.

Mo treatment	Nodules per culture	Dry wt.*	Mo**	Total N
		gm.	*p.p.m.*	*%*
0.015 p.p.m.	31	43.9	8.5	2.01
0.10 p.p.m.	62	40.0	15.0	1.99
None	34	27.6	0.0	1.32
1 microgram†	29	32.0	0.0	1.54
0.001 p.p.m.	53	42.1	0.7	2.07

* Mean yield for three cultures except in the no Mo and one microgram treatment.

** 0.0 = less than 0.1 p.p.m. Mo.

† Immediately after the fifth harvest, this culture was treated with one microgram Mo. Thereafter it received purified solutions containing no Mo.

and contained more total N than those from untreated plants (table III). Additions of Mo to the nutrient solutions produced the same trends in yield and in Mo and N content of roots as were observed for stems and leaves.

Function of molybdenum

Experiments indicate that Mo functions as a biological catalyst in symbiotic-N fixation and in the reduction of nitrates in non-leguminous plants. This study presents evidence that legumes also require Mo for nitrate reduction. The specific reactions catalyzed by Mo are not known. It is possible that accumulation of nitrates in Mo-deficient plants is an indirect effect caused by a lack of Mo in some reaction preliminary to nitrate reduction. Enzyme experiments were conducted on both Mo-deficient and normal alfalfa to study this point.

Young shoots of plants grown after the sixth harvest were ground in a Waring Blendor, extracted with water, and their succinic dehydrogenase activity was estimated by the Thunberg technique (**14**). Five ml. of extract, representing 0.1 gm. of green tissue, from normal plants reduced 1 ml. of 0.00027 M methylene blue in two hours. An equal amount of extract from a deficient plant failed to completely reduce that quantity of methylene blue in 12 hours. Preliminary studies indicated no consistent difference in the rate of reduction of nitrates by nitrate reductase in the two extracts. Since the energy required for nitrate reduction in plants must be supplied by dehydrogenation reactions in respiration, it is concluded that Mo indirectly affects nitrate reduction by enzymatically catalyzing certain dehydrogenation reactions. If this conclusion is correct, then the importance of Mo in Fe metabolism, reported by MILLIKAN (**11**) might be explained. Mo may be a constituent of certain dehydrogenases while Fe in the cytochrome system acts as a hydrogen acceptor for dehydrogenation reactions.

Summary and conclusions

Cultural techniques and a method for the purification of Fe salts were developed for the study of the Mo requirements of alfalfa in nutrient cultures.

Studies were made of the influence of Mo on yield and the Mo and N content of alfalfa grown with purified culture solutions. Four cuttings were supplied with nitrate N and variable amounts of Mo. In the fifth and sixth cuttings, the Mo nutrition of alfalfa was studied when atmospheric N was utilized.

Plants receiving nutrient solutions containing nitrates and no Mo developed deficiency symptoms as indicated by light-green upper leaves and chlorosis and necrosis of lower leaves.

Mo-treated alfalfa supplied with nitrates produced significantly higher yields than the untreated in three of the four cuttings. There was no con-

sistent response to applications of more than 0.001 p.p.m. Mo in nutrient solutions.

Mo-deficient plants contained less total N and they accumulated nitrates when this form of N was supplied.

There was a close relationship between the quantities of Mo applied in nutrient solutions and the Mo content of the tissues. Mo-deficient plants contained less than 0.1 p.p.m. Mo. The leaves consistently contained about 50% more Mo than the stems.

After nitrates were removed from nutrient solutions and the cultures were inoculated, all plants temporarily developed severe N-deficiency symptoms. Within three weeks, the Mo-treated plants regained their normal color and succulence but the untreated plants remained chlorotic. Many upper leaves of deficient plants later showed necrosis of tips and margins while the lower leaves abscissed.

Mo-treated plants grown on atmospheric N produced highly significant increases in yield and contained between 47 and 92% more total N than plants receiving no Mo.

There was no correlation between numbers of nodule masses and yield and N fixation.

The succinic dehydrogenase activity of Mo-deficient plants was much less than that of normal plants supplied with Mo.

The authors wish to express appreciation to Dr. W. R. Robbins for photography and for suggestions during this investigation.

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GROWTH OF WALNUT TREES AS AFFECTED BY IRRIGATION AND NITROGEN DEFICIENCY

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(WITH THREE FIGURES)

Received May 15, 1950

Results obtained in an irrigation experiment with walnuts at the University of California at Davis give some interesting evidence on the problem concerning the availability of soil moisture to plants between the field capacity and the permanent wilting percentage. In 1929 we (**3**) reported on results obtained in an irrigation experiment with peaches and stated that "trees either have readily available soil moisture or have not." In various publications since that time we have reported on results, obtained with other fruits, that point to the same general conclusion—namely, that soil moisture from the field capacity to the permanent wilting percentage is readily available to plants. Physical measurements of the plant soil-moisture relationship substantiate the validity of this conclusion. The lack of water is quickly evidenced by the reduced rate of growth of the fruit; hence data from experiments for one season may yield reliable results. It takes several years, however, to measure the effect of water deficiency upon the growth of the trees.

Other investigators have reported results purporting to show that soil moisture is not equally available throughout the range between the field capacity and the permanent wilting percentage. There have been numerous trials with plants in pots but few with trees in the field. Aldrich, Lewis, and Work in a number of papers (**1, 5, 6, 9**) presented results which they claimed to show that growth of pears was retarded when the soil-moisture content was reduced to somewhere between 50 and 80% of the available supply. These authors, however, pointed out that in the case of the dense clay loam soil used, the root system of the pear trees was sparse and, while the soil might be dry next to the roots, that a short distance away and not occupied by roots might be considerably higher in moisture content. Soil samples taken with a soil tube included both the dry soil next to the roots, and also the moist soil a short distance away, with the average above the permanent wilting percentage. Actually those investigators did not know the moisture content of the soil in contact with the absorbing portion of the tree roots. We have pointed out (**7**) that in case of very dense soils that do not permit the ready entrance of roots such as the clay loam mentioned above the moisture equivalents and permanent wilting percentages as determined on fragmented samples are valueless.

Recently Kenworthy (**4**) published results obtained with apple trees in containers from which he concludes that utilization of 80% or more of

the available moisture significantly reduced shoot growth. His data show no significance at the 5% level between shoot growth when 20% of the moisture was utilized and when 80% was used. The difference was 51 cm. and that required for significance is 59 cm. Reduction of growth when all the moisture content was reduced to the permanent wilting percentage is to be expected. ALLMENDINGER, KENWORTHY and OVERHOLSER (**2**), in similar experiments with apple trees, thought that carbon dioxide intake was reduced when more than 80% of the available soil water had been utilized. Plotting their data for CO_2 intake in mg./hr./dm.^{2} and the soil-moisture content at the time the measurements were made reveal that the difference obtained with the lowest moisture contents and the higher ones is probably not significant.

A recent review (**8**) of the literature on the relation of plant growth to soil moisture shows that plants grow well throughout a wide range of soil moisture but that some investigators question whether they do so with equal facility throughout the entire range from field capacity to permanent wilting percentage.

The results reported in this paper were obtained from an orchard of Concord walnuts on black walnut root planted in 1923. The trees were planted on the square system 48 feet apart. The soil is classified as a Yolo loam with moisture equivalents varying from 22 to 24 and permanent wilting percentages of 11 to 12. The treatments consisted of duplicate plots of six trees each surrounded by guard trees receiving the same irrigation treatment. Except for the irrigation, all plots received the same cultural treatment. No fertilizers were added during the period reported in this paper. The plots were randomized. Differential irrigation was started in 1935. The results presented in this paper were secured from 1935 to 1946 inclusive.

The results from plots in two irrigation treatments are reported in this paper. During the 12-year period, treatment A received four or five irrigations each growing season. The average total amount applied was 33.1 acre-inches per acre or an average of 7.4 inches each application. The average interval between irrigations was 30.6 days. The purpose of this treatment was to keep the soil at a relatively high moisture level. Treatment B received two or three irrigations each season. The average total amount of water was 19.7 inches or 7.9 inches each irrigation. The average interval between irrigations was 44.0 days. Here, the object was to apply water only frequently enough to keep the upper six feet of soil above the permanent wilting percentage. The soil moisture, however, did reach the permanent wilting percentage in July and again in September as pointed out below.

Circumference measurements of the trunks were taken each year and the cross-section areas computed. The yields of hulled nuts from each tree were secured at harvest time.

Results

The soil-moisture record during a typical year is shown in figure 1. Treatment A was irrigated May 26, June 21, July 19 and August 16; and B on June 17 and July 26. The record shows that the moisture was reduced in the top three feet in treatment A to between 15 and 16% on three occasions. Treatment B was reduced to about the permanent wilting percentage late in July and again about the middle of September and remained there for several weeks. In the three- to six-foot depth, the soil moisture in treatment A was reduced to about 18.5% about the middle of July and to about 17.5% early in October. The soil in the three- to six-foot depth in treatment B was reduced to the permanent wilting percentage at about the same time as in the top three feet. The areas between the curves in each diagram indicate the differences that existed in the moisture contents in the two treatments.

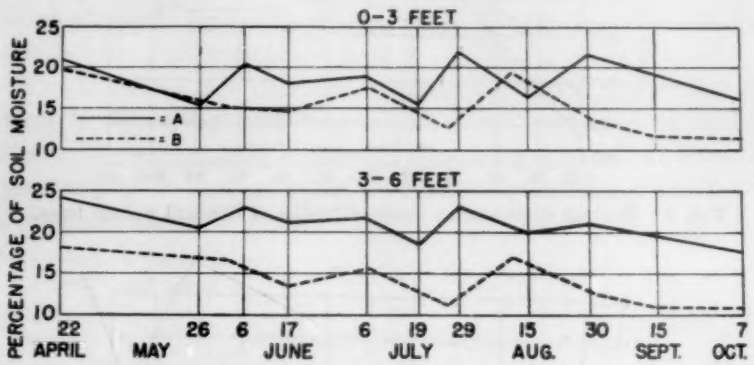

FIG. 1. Soil-moisture record in Concord walnut orchard during a typical year. Solid line, treatment A; dotted line, treatment B.

In field trials involving large plots it is not feasible to irrigate just when the soil moisture has been reduced to certain levels such as 20, 40 or 60% of the available supply. About all that can be done is to allow one treatment to be reduced to about the permanent wilting percentage before irrigating while the other may be maintained at somewhat higher moisture contents. In treatment A, when the moisture was reduced to 15.5%, about 65% of the available supply had been used.

The average cross-section area of the trunks (fig. 2) in 1935, at the beginning of differential irrigation treatment, was 965 sq. cms. in treatment A and 1058 sq. cms. in treatment B. The difference was not significant. Trees in treatment B increased in cross-section areas slightly faster than those in treatment A, in spite of the higher soil-moisture level maintained in treatment A. The difference between treatments B and A became significant at the 5% level in 1943 and remained so through 1946.

The average cumulative yields are shown in figure 3. The yields remained essentially equal until 1944. Since that time the cumulative yields

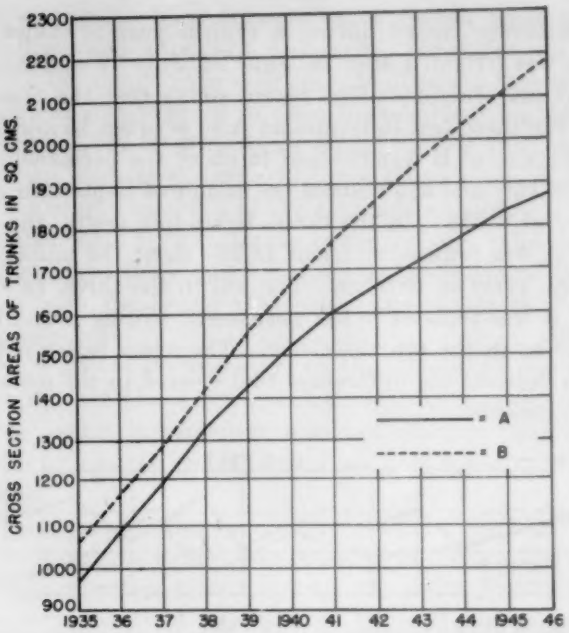

FIG. 2. Average cross-section areas of trunks of Concord walnut trees.

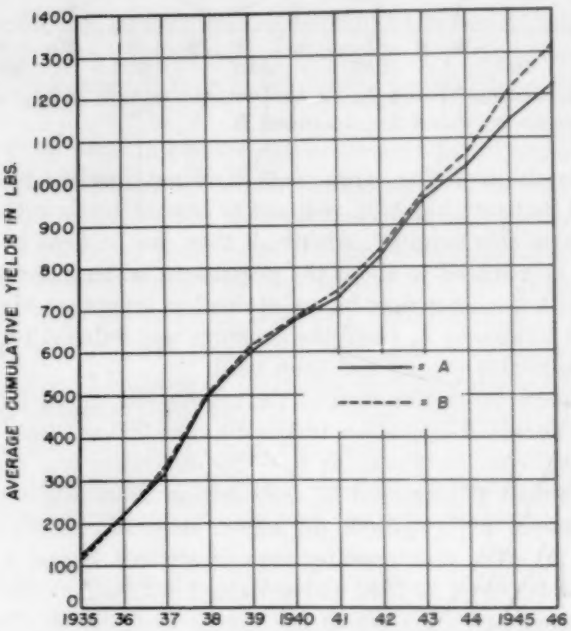

FIG. 3. Average cumulative yields of Concord walnut trees.

in treatment B have been slightly but not significantly larger than those in treatment A.

No explanation for the behavior of treatments A and B was immediately available. At the end of 12 years the trees in treatment A were significantly smaller than those in B, in spite of the fact that in treatment A the soil was kept at a higher moisture level than B.

In 1946 leaf analyses indicated the probable cause for the difference in growth of the trees. The average nitrogen content of the leaves in treatment A was 1.58%, while that in treatment B was 1.84%. The difference was significant at the 5% level. It seems reasonable to assume that a difference in nitrogen content of the leaves of the trees in the two plots existed for a number of years previous to 1946. In other words, the growth of the trees in treatment A was less than that in treatment B because of the lack of an adequate supply of nitrogen even though they were maintained at a comparatively high moisture level.

The results show that nutrition is a factor of great importance in experiments to measure growth responses of plants and must be considered in addition to soil-moisture contents. The main conclusion, however, is that greatest growth does not result from maintaining the soil moisture high in the available range.

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CHELATION OF TRACE METALS IN NUTRIENT SOLUTIONS[1]

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(WITH ONE FIGURE)

Received June 5, 1950

Plant nutrition studies frequently require nearly complete elimination of trace metals from the nutrient media in which the plants are to be grown. Conventional methods of purification by recrystallization and precipitation have been used for this purpose in some studies. These have proved more difficult and less effective than the adsorption technique developed by STEINBERG (**11**) and improved by ARNON and STOUT (**4**), in which heavy metals are coprecipitated with calcium phosphate. In recent years attention has been called to the reaction of heavy metals with certain organic molecules to form chelate complexes which can be removed from nutrient solutions by extraction (**7, 8, 12**). This means of eliminating trace metals from nutrient solutions suggested the possibility that such chelating agents might be used in nutrient solutions in direct contact with the roots of growing plants to provide a continuing control of trace metal contamination in the nutrient media. The present study was undertaken to determine the effect of chelating agents added to nutrient solutions on plants growing in these solutions. Tests reported describe the responses of seedlings of several species and of older tomato plants to six such chelating compounds present in nutrient solutions.

A chelating agent (from the Greek Chele, meaning claw) usually is a complex organic molecule containing two electron-donating groups which, acting similar to the two jaws of a claw, may remove simple metal ions from solution. These chelating reactions are slightly reversible, the degree of reversibility, characterized as the "stability constant," depending on the agent used and the metal ion involved. The mechanism of chelation is illustrated with the following example. Oxine (fig. 1, A), 8-hydroxyquinoline ($HOC_6H_3N:CHCH:CH$), is a double ring compound formed by the fusion (in the ortho position) of a pyridine ring to a benzene ring with the hydroxyl group on the eighth position of the double ring compound. Oxine ionizes to form oxinate and hydrogen ions, the hydrogen coming from the hydroxyl group. The chelating effect of oxine results when the oxygen and nitrogen, each acting as a claw, join to a metal ion (Zn^{++}) thus forming a ring, called a chelate ring. The oxygen, with a free negative valence, forms an electrovalent linkage with the zinc ion while nitrogen forms a co-ordinate covalent bond. Thus the zinc ion is bound securely to the oxine molecule. However, the zinc ion has two free valences and for this reason two molecules of the oxine are required to

[1] Contribution from the Botanical Laboratory, The University of Tennessee, New Ser. no. 119.

bind one zinc ion. The complete structural linkage is diagrammed in figure 1, B. Generally speaking, the above type of complex is formed with any union of heavy metal and chelating agent. Trivalent metals require three molecules of the chelating agent to bind them but the general mechanism is the same. The strength of this association is dependent on several factors (**1**, **2**). Although no definite specificity for any one metal can be ascribed to a particular chelate compound, these compounds do form more stable complexes with some metals than with others.

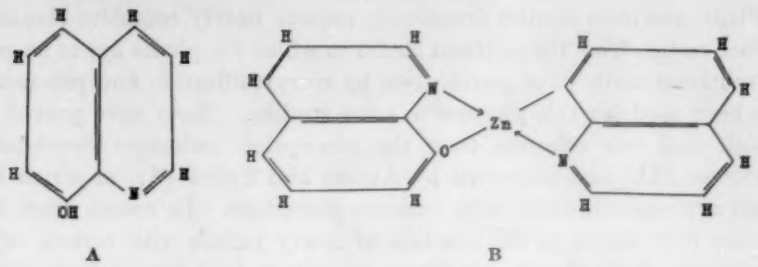

FIG. 1. A, oxine molecule (8-hydroxyquinoline). B, chelate complex of oxine and zinc.

Chelation has been used to some extent as a means of eliminating trace metals from culture solutions. PIPER (**8**) supplemented calcium carbonate coprecipitation of heavy metals from stock solutions with dithizone and chloroform extractions. WARING and WERKMAN (**12**) removed iron from bacterial nutrient solutions by extraction with oxine and chloroform. HEWITT and JONES (**7**) effectively removed traces of molybdenum from stock nutrient solutions by adsorption on a chelate complex. In their procedure oxine was chelated with iron and the molybdenum was removed by coprecipitation with the oxine-iron complex. Several chelating agents used in chlorella cultures have been shown to inhibit photosynthesis and respiration, the inhibitions being reversible upon removal of the chelating agents (**6**). ZENTMYER (**13**) found oxine to be an effective fungistatic agent, but the inhibition of fungal growth could be prevented by adding zinc in amounts sufficient to overcome the chelating action of oxine.

General methods

ALBERT and GLEDSHILL (**1**) investigated 30 chelating agents showing their effects on certain metallic ions at temperatures from 20 to 30° C in the pH range 6.5 to 7.3. Each solution tested was composed of .0001 M metal ions, .05 M borate buffer (pH 7.3) and .001 M chelating agent. Since two molecules of chelating agent combine with one ion of divalent metal the molar concentration of the chelating agent in these solutions was actually five-fold in excess over the divalent metals.

Chemicals used in this study and their reactions with certain metal ions, as determined by Albert and Gledshill, are listed in table I. Salicylic

acid was not characterized by these investigators. Concentration of chelating agents for all tests was based on total metal impurities, estimated at not more than five parts per million or .000078 M metal, computed as copper. Chelating agents, .00078 M, were then supplied to give a five-fold excess in the solutions. This molarity of chelating agents is referred to in all tests as "unit" concentration and all higher molarities used were integral multiples of this unit concentration.

Glass containers used throughout this study were thoroughly washed, then rinsed with tap water, distilled water, concentrated hydrochloric acid, distilled water, and finally redistilled water.

TABLE I

CHELATING AGENTS USED IN THIS STUDY AND THEIR CHELATING REACTION WITH FIVE METAL IONS. AFTER ALBERT AND GLEDSHILL (1)

CHELATING AGENT	MOLECULAR WEIGHT	Mn*	Zn**	Fe**	Fe†	Cu**
Oxine (8-Hydroxyquinoline)	145	**	**	*	*	**
Carbamate (Sodium diethyl-dithiocarbamate)	171	*	†	*	**	†
Chromatropic salt (Sodium 1: 8 dihydroxy-naphthalene 3:6 disul-phonate)	364	†				†
Nitroso-R-salt (Sodium 1-nitroso-2-naphthol 3: 6 disul-phonate)	377			†	**	**
Quinalizarin (Tetrahydroxyanthra-quinone)	272	**	*	*	**	*
Salicylic acid (Hydroxy-benzoic acid)††	138					

* Showed trace chelation with .0001 M metal using .001 M chelating agent
** Showed marked chelation with .0001 M metal using .001 M chelating agent
† Showed trace chelation with .00001 M metal using .001 M chelating agent
†† Not characterized by Albert and Gledshill.

Germination and seedling studies

Germination and seedling tests were designed to study the effects of different chelating agents on root growth in several species of plants. Seeds of tomato, pea, bean, corn, and radish were used in the germination tests. Eight seeds of each species were placed in each of six different solutions: (1) distilled water, (2) carbamate, (3) chromatropic salt, (4) oxine, (5) nitroso-R-salt, (6) quinalizarin. Germination in all solutions, except oxine, was good. Tomato and radish seeds did not germinate in the oxine while the other three species showed no growth after the initial rupture of the seeds.

Seedling studies were made with tomato and corn using nine test solutions (table II). From 200 to 300 seeds of each species were germinated

TABLE II

EFFECT OF CHELATING AGENTS ON GROWTH IN LENGTH OF SEEDLING ROOTS, MEASURED IN CENTIMETERS

PLANTS	REFERENCE SOLUTION*	CARBAMATE 2-FOLD	CARBAMATE 4-FOLD	CARBAMATE 8-FOLD	CHROMATROPIC SALT 2-FOLD
Tomato	4.26	4.21	0.24	0.00	4.27
Corn	16.01	15.12	10.35	4.85	12.18

PLANTS	OXINE 2-FOLD	NITROSO-R-SALT 2-FOLD	QUINALIZARIN 2-FOLD	SALICYLIC ACID 2-FOLD
Tomato	0.00	4.23	4.21	2.99
Corn	2.54	14.68	17.13	14.20

* The reference solution served as a standard of comparison for all other test solutions which contained the chelating agents as well as the basic nutrients.

in Petri dishes using distilled water. Eight corn and 15 tomato seedlings, selected for uniform growth, were transferred to large culture dishes containing the test solutions. After three days for corn, and seven days for tomato, the length of the primary roots was determined as a measure of the effect of the chelating agent on growth. The results (table II) were in agreement with those obtained with germinating seeds and showed that oxine was injurious to root growth of both species, the roots turning brown in less than twelve hours. Both species showed root growth to be inversely related to the carbamate concentration; however, the roots were not discolored and remained turgid in the two lower carbamate concentrations and only half of the roots in the eight-fold concentration were dead. Roots in chromatropic salt, nitroso-R-salt, quinalizarin, and salicylic acid showed growth comparable to that in the reference solution, as did the carbamate at two-fold concentration.

Water culture studies

Tomato, variety Marglobe, was the major test plant in these studies. Plants were germinated in white sand and transferred to 125 ml. flasks containing basic nutrients plus boron, manganese, zinc, and copper. Roots were washed with distilled water and rinsed with double distilled water before being placed in test solutions. Early tests were made in 500 ml. flasks without aeration, while quart Mason jars, covered with brown paper and aerated continuously, were used in later tests. Solutions were renewed every three weeks, and distilled water was added regularly to avoid any major change in the concentration of chelating agents. Stock solu-

tions of each chelating agent, with the basic nutrient salts (3) and 0.286% H_3BO_3, were stored in two-liter Pyrex flasks. A stock iron solution of 0.5% $FeCl_3$ and 0.5% tartaric acid and a stock supplementary solution, containing 0.181% $MnCl_2 \cdot 4H_2O$, 0.0222% $ZnCO_4 \cdot 7H_2O$, and 0.0079% $CuSO_4 \cdot 5H_2O$, were prepared so that one ml. of each could be added to a liter of test solution, where desired, to make a complete nutrient solution.

Since chelating agents were used in the nutrient solutions, heavy metals, such as iron, manganese, zinc, and copper, had to be supplied to the plants by a method other than direct addition to the nutrient solution. Spraying with a 0.3% iron solution resulted in poor chlorophyll distribution, the leaves having mottled appearance. This condition was not improved materially by the addition of a little Vel to the spray solution. Since a uniform green color is essential for recognition of various other deficiencies, the spray technique was discarded in favor of the injection method. This procedure, developed by ROACH (9) and ROBERTS (10) and modified by FELBER (5), permitted injection for a period of time. Eight strands of cotton darning thread were soaked in the injection solution and threaded through the stem. One end of the thread dipped into a reservoir of this solution in a small vial tied to the stem. Two injection solutions were used: (1) 0.1% iron, as ferrous ammonium sulphate, acidified with HCl to pH 1.5; (2) 0.1% iron and the supplementary solution, containing manganese, zinc, and copper, at one fifth the concentration of the nutrient stock solution or 200 times the concentration actually used in the nutrient solution. In most cases a single injection of one ml. was sufficient for six to eight weeks' growth. Best results were obtained by injecting immediately after threading under conditions favorable for high transpiration. With these conditions one ml. could be injected and distributed throughout the plant within four hours. Slow injection for eight to 10 hours resulted in considerable injury at the point of injection of the stem. If more than one ml. was injected, toxicity was evident in some leaves and along the stem directly above the point of injection.

Chelating agents used in the water culture tests were the same as in previous studies with seeds and seedlings (table III). However, in these tests with older plants a number of concentrations of some of the agents were used. Oxine, which was used successfully in bacterial studies by WARING and WERKMAN (12), was included in these studies, even though seeds and seedlings failed to survive in oxine solutions. Carbamate was studied more extensively than any of the other chelating agents because of the number of metals chelated and the tenacity with which some are held.

Tomato, bean, corn, and Coleus were used in the oxine studies. No growth was evident in any of these plants (table III). The roots died quickly and no adventitious roots formed. The lower stems of all plants, except corn, became constricted to a thin black band. This constriction gradually extended up the stems until, in some cases, even the lower petioles showed constriction. Tops remained turgid throughout the tests

TABLE III

GROWTH AND DEFICIENCY RESPONSES OF PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARIOUS CHELATING AGENTS ADDED

TEST SOLUTIONS	NO TRACE METALS ADDED	Fe, Mn, Zn, Cu ADDED TO TEST SOLUTION	Fe, Mn, Zn, Cu INJECTED INTO PLANTS	Fe INJECTED INTO PLANTS
Reference solution	Fe deficiency	Good growth*	Good growth	Mn deficiency
Oxine	No growth	No growth		
Oxine, two-fold	No growth	No growth		
Carbamate	Fe deficiency	Fe deficiency		
Carbamate, two-fold	Fe deficiency	Fe deficiency	Good growth	Mn deficiency
Carbamate, four-fold	Little growth	Little growth	Little growth	Little growth
Carbamate, eight-fold	Little growth			
Carbamate, twelve-fold	No growth			
Carbamate, sixteen-fold	No growth			
Chromatropic salt	Fe deficiency	Good growth		
Chromatropic salt, two-fold	Fe deficiency	Good growth	Good growth	Fair growth
Chromatropic salt, three-fold	Little growth			
Chromatropic salt, four-fold	Little growth			
Nitroso-R-salt	Fe deficiency	Fe deficiency		
Nitroso-R-salt, two-fold			Fair growth	Little growth
Quinalizarin	Fe deficiency	Fe deficiency		
Quinalizarin, two-fold			Good growth	Mn deficiency
Salicylic acid, two-fold	Fe deficiency	Good growth	Fair growth	Fair growth

* Plants grown in complete nutrient solution without chelating agents were regarded as growth standards.

but showed no growth in six weeks. The leaves showed marginal necrosis which gradually included the whole leaf.

The other five chelating agents (table III) showed no deleterious effects on growth of tomato plants up to two-fold concentration of the agents. Iron deficiencies were characteristic in those tests which received no trace metal additions either through the test solution or by injection. When iron and supplementary solutions were added to the test solution an iron deficiency was still produced in all cases except with chromatropic salt and salicylic acid, which did not chelate iron effectively. Injection of iron and supplementary solutions corrected iron deficiencies in all cases. When the iron deficiency was corrected by injection of iron alone a manganese deficiency appeared in carbamate and quinalizarin tests within four weeks, and reduced growth was noted in the plants in chromatropic salt. Since the manganese deficiency was also apparent in the reference solution one ml. of the supplementary solution was added to those solutions containing manganese-deficient plants. Within four days partial recovery was noted in all cases, regardless of the presence of chelating agents. Within a week all plants in these tests had recovered completely.

Increasing the concentration of chromatropic salt and carbamate in the nutrient solutions (table III) failed to show injury effects which could be identified as specific nutrient disorders. Roots died, lower parts of the stems became constricted, eventually collapsing, and the leaves showed marginal necrotic areas. These injury effects were quite similar to those evident in oxine tests. The rate of injury, at concentrations over two-fold, was directly related to the molarity of the chelating agent up to concentrations of four-fold chromatropic salt and 16-fold carbamate.

Studies were made to determine the nature of the injury caused by oxine and high concentrations of carbamate. The root system of a tomato plant was split lengthwise, half being placed in an oxine solution and the other half in a complete nutrient solution. The roots in oxine died while a good root system was developed in the complete nutrient solution. The plant itself showed good growth in the six weeks it was under test. These results indicate that oxine effects are due to root injury and oxine is not translocated to upper parts of the plant.

ZENTMYER (**13**) obtained good fungus growth in oxine after adding sufficient zinc to complex all the oxine. This was tried in these studies using iron to complex oxine and zinc to complex the 16-fold carbamate. The plants in oxine showed good growth while the plants in 16-fold carbamate showed fair growth, thus agreeing with Zentmyer's results and indicating that the root injury from oxine and carbamate was caused by the chelating action of these two agents.

Discussion

The use of chelation for removal of trace metals in nutrient studies with higher plants has not been explored extensively. Extractions with dithi-

zone and oxine have been used in a few instances as a means of removing heavy metals from stock solutions (**7, 8, 12**). The present study was undertaken to determine if plants could be grown directly in solutions containing chelating agents, thus providing continuing control of heavy metal contamination.

The results of this study indicate that the effectiveness of chelation determines whether plants can grow in solutions containing chelating agents. At low concentrations of all the agents used, other than oxine, chelation was ineffective except for iron in some cases. When iron was added by injection to plants growing in solutions where iron was effectively chelated, plant growth was good. Chelation is a reversible process, the degree of dissociation (stability constant) depending on the agent used and the metal ion involved. Since the unit molarity of chelating agent gave a five-fold excess over the divalent metal ions, these agents, other than oxine, must dissociate appreciably. Consequently, sufficient metal was left in solution in these lower concentrations of chelating agent to provide for good plant growth, except for iron in some cases.

Oxine at two-fold concentration, and carbamate and chromatropic salt at higher concentrations, produce general and severe plant injury. ZENTMYER (**13**) attributed the fungistatic mechanism of oxine to its ability to remove zinc from the culture medium. Quite likely root injury involves this mechanism also. Apparently these chelating agents complex the metals so effectively that their concentration in the solution is infinitely small, resulting in outward diffusion of the metals from the roots into the solution and consequent death of the root cells. Entrance of the chelating agents into the root cells is not likely since the tomato plant with the divided root system thrived even though half of the roots were immersed in the oxine solution.

Results of this study indicate that chelation of heavy metal ions in the nutrient solution of growing plants is not feasible. There seems to be a fairly critical point below which chelation is not effective and above which chelation is effective and plants are injured. This point will vary according to the stability constant for a given reagent and the concentration of metal, as impurities, in any one solution used. In fact concentrations of agents in the solutions vary greatly as water is lost from and replaced to the solutions. Presumably all chelating agents would react similarly, since the chelating mechanism is the same in all cases.

Removal of heavy metal ions in nutrient solutions by extraction with chelating agents (**7, 12**) appears to be a more promising method of purification. Oxine at low concentrations and carbamate at high concentrations should give very good results. Since calcium and magnesium are not chelated at pH 6.5 to 7.3 (**1**), the four stock solutions composing the basic nutrient solution (**3**) could be treated separately with either oxine or carbamate and the chelate-metal complexes then could be removed by extraction with chloroform. Additional studies are planned to test these and

other suggestions concerning the use of procedures involving extraction of chelate-metal complexes as a means of removing trace metals from nutrient solutions.

Summary

1. The effect of chelating agents added to nutrient solutions on plants growing in these solutions was investigated. Chelating chemicals used were: 8-hydroxyquinoline (oxine), sodium diethyldithiocarbamate (carbamate), sodium 1:8 dihydroxynaphthalene 3:6 disulphonate (chromatropic salt), sodium 1-nitroso-2-naphthol 3:6 disulphonate (nitroso-R-salt), tetrahydroxyanthraquinone (quinalizarin), and hydroxy-benzoic acid (salicylic acid).

2. Germination and seedling studies with tomato, pea, bean, corn, and radish, and water culture tests with tomatoes, using various concentrations of the chelating agents, are described, and plant responses to these reagents examined.

3. Iron was the only metal ion effectively removed from solution at low concentrations of oxine, carbamate, nitroso-R-salt, and quinalizarin. Iron was not effectively chelated by either chromatropic salt or salicylic acid.

4. Concentrations of chelating agents in which plants would grow did not chelate metal ions effectively, while concentrations of chelating agents which chelated effectively produced severe plant injury. This injury resulted from heavy metals being extracted from the root cells.

5. Since effective chelation in the nutrient solution results in severe plant injury, the use of such agents in nutrient solutions in direct contact with the roots of growing plants is not a feasible method of controlling trace metal contamination in such solutions.

The authors are pleased to acknowledge technical assistance of Dr. G. K. Schweitzer on chelation aspects of this study.

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INHIBITION OF CURVATURE RESPONSES BY SHUNTING THE INHERENT ELECTRICAL FIELD[1]

A. R. Schrank

(WITH FOUR FIGURES)

Received March 4, 1950

Introduction

In studies of the bioelectric fields of the onion root, Rosene (**2**) found that the magnitude of the longitudinal polarity was decreased or shunted when a jacket of tap water was placed around a segment of the root between the contacts. When the shunt was removed, the polarity quickly returned to practically its former level. Similar results were obtained by Wilks and Lund (**7**) from their experiments with the Avena coleoptile. They observed that filling isolated sheaths (taken from 34 to 36 millimeter seedlings) with Shive's solution caused the magnitude of the external longitudinal polarity to be decreased from 40 to 60 to less than 10 millivolts. As in the onion root, when the solution was removed from the inside of the sheath, the electrical polarity was promptly restored. These facts were presented as proof of internal shunting of some of the polar cells which contribute to the longitudinal electrical polarity of the sheath.

Wilks and Lund (**7**) also reported preliminary experiments in which the central cavities of isolated coleoptiles were filled with various solutions and then exposed unilaterally to 400 meter candles of white light. Coleoptiles that were filled with Shive's solution did not show any bending response, and those filled with distilled water curved only slightly when compared to the curvature of the air-filled sheaths. Since it is known from previous data that electrolytic solutions, such as Shive's, short-circuit the electrical polarity, these experiments indicate that the inherent electrical field is a fundamental prerequisite for the growth curvature processes.

The purpose of the present paper is to present supplementary data which show the quantitative effects of short-circuiting the inherent electrical field, using different concentrations of Shive's solution, on the various tropic responses of the Avena coleoptile.

Experimental procedure

Isolated coleoptiles of *Avena sativa*, Victory Strain (U. S. Department of Agriculture C. I. 2020) were used in all experiments. Etiolated seedlings were grown and prepared in the manner described in previous publications (**3, 4**). The laboratory conditions and the apparatus used were also the same as in preceding investigations (**3, 4**).

The coleoptile sheaths which were used in these experiments were isolated from intact seedlings that were 30 ($\pm$ 1) millimeters long, perfectly

[1] Supported by the University of Texas Research Institute.

straight, and had a well-developed root system. A cut was made all the way around the coleoptile about five millimeters apical to the seed so that the primary leaves could be pulled out of the basal end. The hollow coleoptile cylinder was next filled with one of the solutions. Either a glass pipette with a capillary tip or a hypodermic syringe with a blunt needle was used for the filling operation. The sheath was then placed in a glass holder with its cut end submerged in Shive's solution (**6**)[2] and permitted to remain undisturbed for one hour to recover from the stimulation of handling. At the end of this time one of the several types of stimuli was applied. After stimulation, growth curvature was measured by following the tip position with an ocular micrometer. At the end of each experiment the total angular curvature was determined by the Judkins method (**1**). The techniques for making the electrical measurements have been published in preceding papers (**3, 4**).

Results

Stimulation by gravity

In this first series of experiments the coleoptiles were stimulated by placing them in the horizontal position for one hour. The upward curvatures that were observed are shown in figure 1, in which each curve represents an average of six or more experiments. The initial downward curvature, common in all of the curves, is due to the weight of the coleoptiles.

Curve I shows the average curvature of the sheaths which were not filled with any solution so that only air occupied the inside space. This curve serves as a sort of control indicating the maximum geotropic curvature that was observed under these conditions. Curves II, III and IV were obtained from sheaths filled with Shive's solution X $\frac{1}{2}$, X 1 and X 2, respectively. Sheaths filled with Shive's solution X 2 show only slightly more than half as much curvature as air-filled coleoptiles. The curves in figure 1 clearly show that both the rate and the final angular geotropic curvature of isolated sheaths are inhibited by Shive's solution, and that the extent of the inhibition is dependent on the concentration of the electrolyte with which the cylinder was filled.

Since the higher concentrations of Shive's solution inhibit geotropic bending most effectively, there is the possibility that the inhibition could be mediated by osmotic mechanisms. Results of an additional series of experiments, designed to test this possibility, are presented in figure 1 by curve VI. In this series the isolated cylinders were filled with a solution of glycerol in distilled water, with the concentration selected to give an osmotic pressure of 3.5 atmospheres. This should be at least equal to the pressure of Shive's solution X 2, which was the most concentrated electrolyte used in any of the present experiments. It is evident from the comparison of curves VI and I that the glycerol solution on the inside of the cylinder in no way limits the upward bending of the isolated coleoptile.

[2] Shive's solution R5S2 was used in all experiments.

Table I gives the final angular curvatures of the individual experiments in order to illustrate the uniformity of the results. The numerals were arranged in descending order to facilitate making the comparisons.

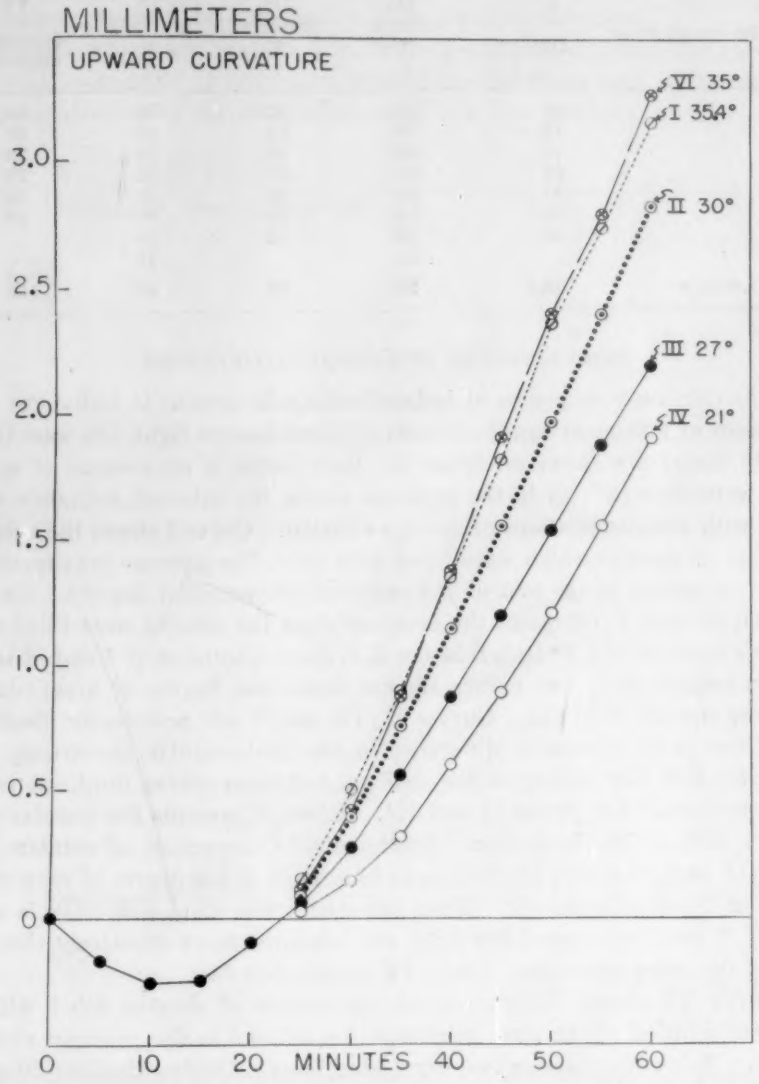

FIG. 1. Upward curvatures of isolated Avena coleoptiles which were placed in the horizontal position for 60 minutes. Each curve represents an average of six or more experiments. Internal cylinders were filled with the following:

Curve I, Air
Curve II, Shive's solution X ½
Curve III, Shive's solution X 1
Curve IV, Shive's solution X 2
Curve VI, Glycerol solution

TABLE I

DEGREES OF UPWARD CURVATURE OF ISOLATED AVENA COLEOPTILES WHICH WERE FILLED AS INDICATED AND KEPT IN THE HORIZONTAL POSITION FOR 60 MINUTES

SHEATH FILLED WITH	I AIR	II SHIVE'S X ½	III SHIVE'S X 1	IV SHIVE'S X 2	VI GLYCEROL SOLUTION
	42	39	32	30	38
	42	36	31	26	37
	40	35	29	24	36
	35	31	27	22	35
	31	30	24	21	34
	29	28	23	20	30
	29	26	22	14	
		15		11	
Average	35.4	30	27	21	35

STIMULATION BY UNILATERAL ILLUMINATION

The curvature responses of isolated coleoptile sheaths to unilateral illumination of 200-meter-candle-seconds of incandescent light (10 watt G. E. Mazda lamp) are shown in figure 2. Each curve is an average of six or more experiments. As in the previous series, the internal cylinders were filled with various dilutions of Shive's solution. Curve I shows the average bending of sheaths which were filled with air. The average maximum angular curvature at the end of the experiments was 16.3 degrees. Curves II, III, IV and V represent the bending when the sheaths were filled with Shive's solution X ½, Shive's solution X 1, Shive's solution X 2 and distilled water, respectively. All curves show a significant degree of inhibition of bending toward the light. Curves II, III and V are practically identical, and there is no expressive difference in the final angular curvatures. In order to check the validity of the similarity of these curves, duplicate series were performed for curves II and III. Table II presents the angular curvature data of the individual experiments. Comparison of column IIA with IIB and IIIA with IIIB gives an indication of the degree of reproducibility of these experiments. When the sheath was filled with Shive's solution X 2, bending toward the light was inhibited more effectively than by any of the other solutions. Curve IV reveals this fact.

Curve VI shows the phototropic curvature of sheaths filled with a glycerol solution of the same concentration as used in the previous experiments. This curve reveals two surprising facts. During the first 80 minutes the average bending toward the light is slightly greater than it is for the air filled coleoptiles, and appreciably more than for the sheaths filled with distilled water. The inhibitory effect of the distilled water on bending toward the light seems to be completely masked by the presence of the glycerol. Data that are available do not warrant an explanation of this observation. These results substantiate a previous suggestion that the inhibition of curvature caused by various concentrations of Shive's solution

apparently is not directly dependent on an osmotic factor. The second feature of curve VI is that the curvature practically stops after 80 minutes. This type of response is not apparent in any of the other curves. The presence of the glycerol limits the maximum curvature attainable by stopping the bending toward the light earlier than the other solutions.

Results, which are presented in figure 2 and table II, indicate that the rate and magnitude of curvature toward the light are both influenced by the concentration of the electrolyte solution in the internal cylinder. The

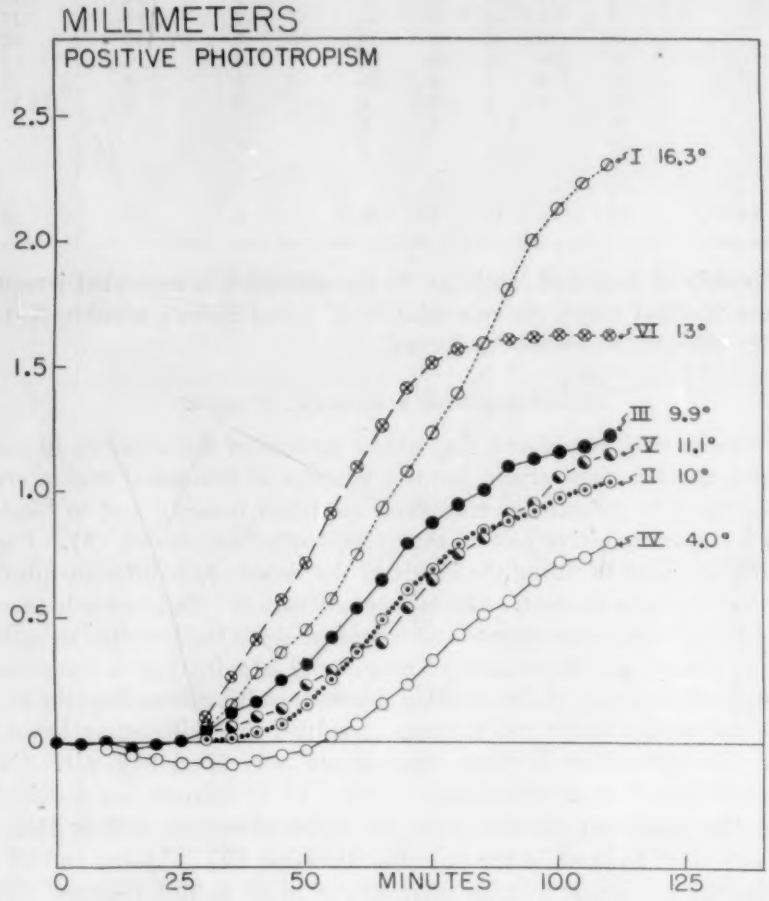

FIG. 2. Curvatures of isolated Avena coleoptiles toward 200-meter-candle-seconds of unilateral illumination. Each curve represents an average of six or more experiments. Internal cylinders were filled with the following:

Curve I, Air
Curve II, Shive's solution X ½
Curve III, Shive's solution X 1
Curve IV, Shive's solution X 2
Curve V, Distilled water
Curve VI, Glycerol solution

TABLE II

DEGREES OF CURVATURE OF ISOLATED AVENA COLEOPTILES WHICH WERE FILLED AS INDICATED AND STIMULATED BY 200-METER-CANDLE-SECONDS OF UNILATERAL ILLUMINATION

SHEATH FILLED WITH	I AIR	II SHIVE'S X ½		III SHIVE'S X 1		IV SHIVE'S X 2	V DISTILLED WATER	VI GLYCEROL SOLUTION
		A	B	A	B			
	20	12	14	13	17	9	14	17
	18	11	12	11	15	7	12	15
	18	10	12	11	13	6	12	13
	17	10	11	10	13	4	11	12
	16	9	9	9	10	0	9	11
	16	8	9	8	8	−2	9	10
	16			7	8			
	16				6			
	15				5			
	11							
Average	16.3	10	11	9.9	10.5	4	11.1	13

relationship of degree of inhibition to concentration is somewhat irregular, because distilled water, Shive's solution X ½ and Shive's solution X 1 are equally effective curvature inhibitors.

STIMULATION BY ELECTRICAL CURRENT

Previous work has shown that direct current of the order of 10 microamperes applied transversely for two minutes to the apical region causes the coleoptile to establish a transverse electrical polarity and to bend toward the electropositive pole of the current applying circuit (**5**). Figure 3 shows the effect of filling the inside of the sheath with different dilutions of Shive's solution on electrically induced curvature. Each curve is an average of five or more experiments and is plotted above the zero line to indicate bending toward the electropositive pole of the circuit. Curve I represents the temporal sequence of the bending process resulting from the application of 10 microamperes for two minutes at a level five millimeters below the apex. The coleoptiles in these experiments were filled with air. Under the conditions of these experiments (Curve I) 70 minutes are required to attain the maximum curvature in the initial direction. After this, the seedlings start to bend in the opposite direction (**5**). At the end of 110 minutes the remaining average angular curvature is 10.1 degrees. When the coleoptile is filled with distilled water and stimulated electrically (Curve V), bending in the first direction is initiated more slowly. The maximum curvature that is reached is about the same as shown by curve I, but an additional 25 minutes are required to reach this maximum. Because of this fact the average angular curvature in the initial direction at the end of 110 minutes is larger than the curvature corresponding to curve I. Shive's solution X ½ inhibits the rate and magnitude of bending toward the

electropositive pole of the current-applying circuit. This fact is apparent from curve II. Filling the inside cylinder of the coleoptile with Shive's solution X 1 and Shive's solution X 2 inhibits electrically induced bending more effectively than Shive's solutions X $\frac{1}{2}$ or distilled water. This is made evident by comparing curves III and IV to curves II and V. It is

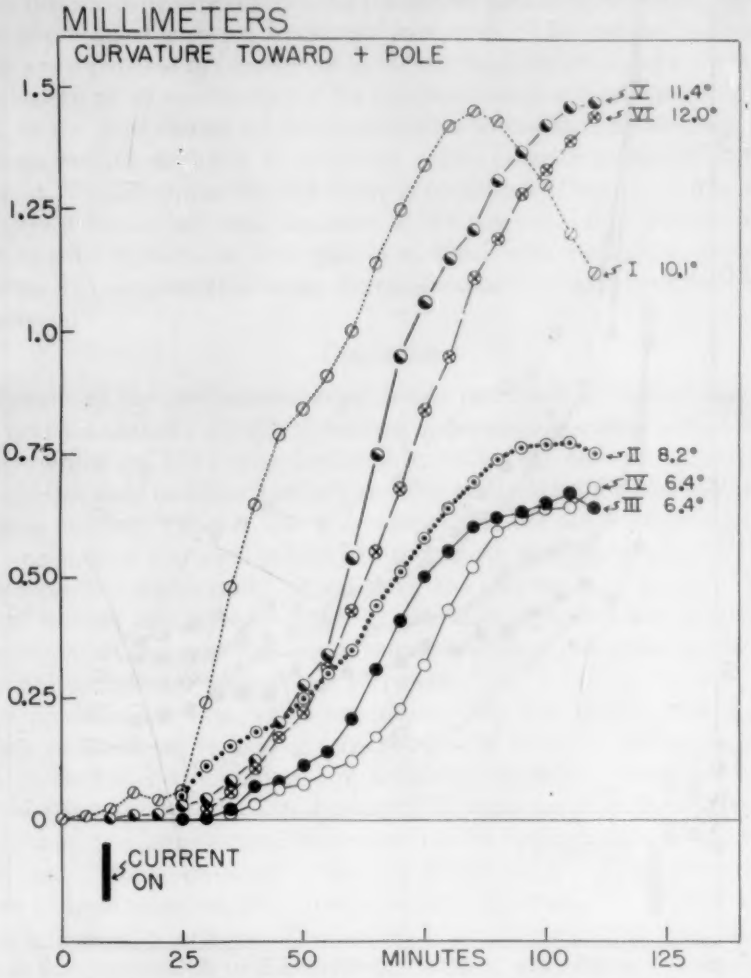

FIG. 3. Curvatures of isolated Avena coleoptiles induced by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters below the apex. Each curve represents an average of five or more experiments. Internal cylinders were filled with the following:

Curve I, Air
Curve II, Shive's solution X $\frac{1}{2}$
Curve III, Shive's solution X 1
Curve IV, Shive's solution X 2
Curve V, Distilled water
Curve VI, Glycerol solution

noted that curves I, II, III and IV reach the maximum in about the same time. From this it appears that the time required to attain the upper limit

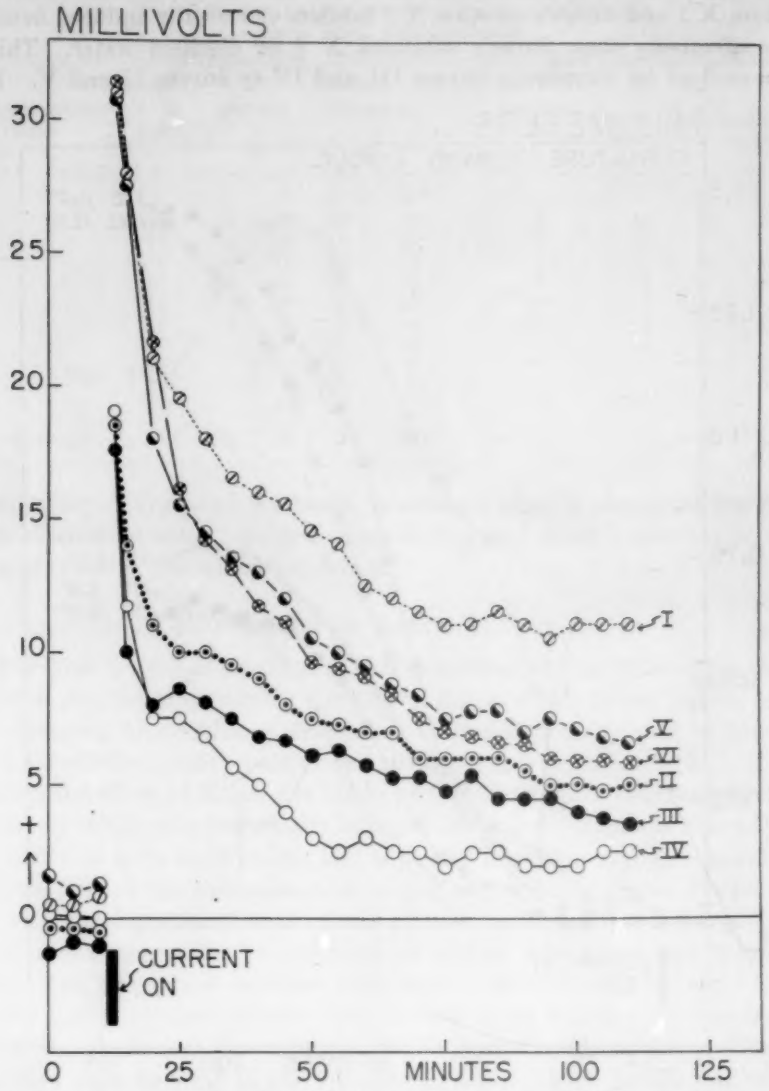

FIG. 4. Transverse electrical polarities established in isolated Avena coleoptiles by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters below the apex. Each curve represents an average of five or more experiments. Internal cylinders were filled with the following:

Curve I, Air
Curve II, Shive's solution X $\frac{1}{2}$
Curve III, Shive's solution X 1
Curve IV, Shive's solution X 2
Curve VI, Distilled water
Curve V, Glycerol solution

of curvature in the initial direction is not dependent on the concentration of the solution with which the cylinders were filled. Curvature responses of coleoptiles filled with the glycerol solution (Curve VI) are practically identical to the responses of sheaths filled with distilled water. This indicates that glycerol is entirely without effect on electrotropic bending.

In this series of experiments the transverse electrical responses resulting from the applied current were also measured. The curves in figure 4, which are numbered to correspond to the curves in figure 3, show the results. Application of 10 microamperes for two minutes at a level five millimeters basal to the apex causes air-filled coleoptiles to establish an average transverse electrical polarity of 31 millivolts. This polarity gradually decreases to about 10 millivolts at the end of the experiments (Curve I). The curves in figure 4 fall in the same sequence as the corresponding bending curves in figure 3. Coleoptiles that had been filled with Shive's solution X 2 manifest the smallest transverse electrical polarity throughout most of the experiment.

Discussion

Results of the preliminary experiments published by WILKS and LUND (7) indicate that the growth curvature responses of isolated Avena coleoptiles to unilateral light were inhibited by filling the internal cylinder with electrolytes such as Shive's solution. Non-electrolytes did not inhibit phototropic bending. It was also demonstrated by the same investigators that the longitudinal electrical polarity of the sheath disappears when it is filled with a conducting solution. Apparently the electromotive forces of the internal surface are short-circuited by the electrolyte. These results were interpreted to indicate that the inherent electrical potentials are a necessary prerequisite for phototropic responses.

Supplementary data, which are presented in this paper, show that curvature responses of isolated Avena sheaths to light, as well as to gravity and to electrical stimulation, are definitely inhibited when the internal cylinder is filled with Shive's solution. It turns out that the degree of inhibition is, to a large extent, dependent on the concentration of the electrolyte used. There are some differences in the degree of effectiveness of a given concentration on the various growth responses, but Shive's solution X 2 is generally a more effective inhibitor of curvature than the more dilute components. Furthermore, the present experiments show that the magnitude of the transverse electrical polarity, which is induced by the applied direct current, is also lowered when the sheath is filled with Shive's solution. The extent of the inhibition of this polarity is again a function of the solution concentration.

One of the first questions to be answered would be whether or not the various concentrations of salt solution exert their effect on growth by altering the osmotic relations within the cells. Control experiments were included in which the cylinders were filled with a water solution of glycerol.

Glycerol was selected because it is essentially a non-electrolyte,[3] and the concentration was adjusted to exceed the maximum osmotic pressure of Shive's solution X 2. The results show that the glycerol solution did not inhibit the curvature in any one of the series of these experiments. One tentative explanation of this would be that a decrease in turgidity of the tissue is not responsible for the inhibition of the bending by Shive's solution. Such interpretation, however, involves the question of permeability of the cell membranes to glycerol as compared to the inorganic salts in Shive's solution, and therefore must be made with caution. More data are obviously necessary for the clarification of this point.

It is also clear from figure 4 that glycerol has no effect on the transverse electrical polarity which is induced by applied current, while Shive's solution does. Thus the present results are consistent with the thesis that the inherent electrical field is an essential requirement for the various curvature responses, and that a decrease in these polarities by shunting them always results in less bending.

Summary

1. Geotropic curvature of isolated Avena coleoptiles is inhibited by filling the internal cylinder of the sheath with an electrolyte, Shive's solution. The degree of inhibition is directly dependent on the concentration of the solution used.

2. Bending toward 200-meter-candle-seconds of unilateral illumination is also inhibited by filling the sheath with Shive's solution. Shive's solution X 2 causes most inhibition, while distilled water, Shive's solution X ½ and X 1 are about equally effective.

3. Similarly, filling the isolated coleoptiles with Shive's solution inhibits the curvature induced by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters basal to the apex. The degree of inhibition is, to some extent, dependent on the concentration of the solution. The transverse electrical polarity induced by current, applied as indicated, is also decreased by Shive's solution; the degree of inhibition is dependent on the concentration used. Glycerol (14.4 grams per liter of distilled water) has no effect on the transverse electrical polarity induced by direct current.

4. Growth curvature responses of the coleoptile sheath to stimulation by gravity, unilateral illumination, and transversely applied current are not inhibited when the cylinders are filled with a solution of non-conducting glycerol with the concentration adjusted to exceed the osmotic pressure of Shive's solution X 2.

5. Previous investigations have shown that the longitudinal electrical polarity of the Avena coleoptile can be shunted by filling the sheath with an electrolyte. Since the present experiments show that the use of elec-

[3] The specific conductances (measured at 25° C.) of the various solutions used are as follows: Distilled water, 0.0000093; Shive's solution X ½, 0.0023; Shive's solution X 1, 0.0042; Shive's solution X 2, 0.0075; 0.145 M glycerol solution, 0.000024.

trolytes always results in less curvature, they support the hypothesis that the existence of an inherent electrical field is an essential requirement for growth responses.

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GROWTH OF *ANANAS COMOSUS* (L.) MERR. AT DIFFERENT LEVELS OF MINERAL NUTRITION UNDER GREENHOUSE AND FIELD CONDITIONS. I. PLANT AND FRUIT WEIGHTS AND ABSORPTION OF NITRATE AND POTASSIUM AT DIFFERENT GROWTH INTERVALS[1]

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(WITH EIGHT FIGURES)

Received February 24, 1950

Introduction

Pineapple plants, fertilized under field conditions with ammonium sulphate, may absorb from the soil ammonium or nitrate or both simultaneously due to the oxidation and conversion of the former to the latter by micro-biological activity. Following absorption by the roots, ammonium is converted to amide, amino, or protein compounds, but nitrate is translocated *per se* via the stem to the leaves, where in the chlorophyllous but not in the non-chlorophyllous tissues it is assimilated by conversion to proteins and other related compounds (**12**).

Available data (**12**) indicate that ammonium is absorbed by pineapple roots at much greater rates than nitrate. However, because of the conversion of NH_4 to NO_3 in the soil within a few weeks after the time of application under favorable conditions, the absorption of nitrogen by the roots as nitrate may be greater than as ammonium, due to longer periods of contact of the former with the roots.

The assimilation of nitrate in the chlorophyllous but not in the non-chlorophyllous basal sections of the leaves makes possible chemical determinations of the nitrate concentrations in the latter sections which may be made the basis, as to amounts and time, for future applications of nitrogenous fertilizers.

The results reported below furnish information, at different growth intervals, on the weights of the plants, leaves, stems, peduncle and fruit, and concentrations of nitrate in the same organs of cultures grown in nutrient solutions in the greenhouse and in the soil in the field, with different amounts of nitrogen, potassium and calcium.

Methods

Cultural

Crowns (vegetative organs produced at the apical end of fruits) from a single clone of uniform weight, length of leaves and thickness of stem, were selected and suspended, for approximately four weeks, through a

[1] Published with the approval of the Director as Technical Paper No. 192 of the Pineapple Research Institute, University of Hawaii.

3-inch hole in a board 12″ × 12″ × 1″ over a 4-gallon porcelain crock filled with tap water for root initiation and development. At the end of this period, the plants with uniformly developed root systems were selected and grown subsequently in nutrient solutions of different concentrations of nitrate, potassium and calcium, while all other nutrient elements were kept at relatively constant levels, as in table I.

TABLE I

MOLAL CONCENTRATION OF DIFFERENT SALTS IN THE VARIOUS CULTURES

SALTS	CULTURES								
	A	B	C OR H	D	E	F	G	I	J
$Ca(NO_3)_2$	.0005	.001	.002	.004	.005	.0005	.001	.002	.002
$CaSO_4 2H_2O$	.....	.....	.....	.....	.....	.0016	.0011	.....	.....
K_2SO_4	.001	.001	.001	.001	.001	.00025	.0005	.....	.....
KNO_3	.....	.....	.....	.....	.....	.....	.....	.004	.006
$MgSO_4$	.001	.001	.001	.001	.001	.001	.001	.001	.001
NaH_2PO_4	.00025	.00025	.00025	.00025	.00025	.00025	.00025	.00025	.00025
$Fe\ SO_4$	.00005	.00005	.00005	.00005	.00005	.00005	.00005	.00005	.00005
$ZnSO_4$	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001
$Na_2B_4O_7$	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001

Concentrations in milligrams per liter of solution of NO_3 and Ca for cultures A, B, C, D, and E were, respectively, 14 and 20, 28 and 40, 56 and 80, 112 and 160, and 140 and 195, with K constant at 78 γ. Similar concentrations of NO_3 and K for cultures F, G, H, I, and J were respectively 14 and 19, 28 and 39, 56 and 78, 112 and 156, and 140 and 234, with Ca constant at 80 γ.

All sets, each comprising 16 plants with one plant per crock, were constantly aerated and the nutrient solutions changed at 3-week intervals. At the time of changing the nutrient solutions, aliquot samples were taken of the old solutions before discarding and of the new ones before transferring the plants thereto, and measurements were made for nitrate, potassium and calcium. Also, volume measurements of these solutions at the beginning and end of intervals of change were made and adjusted to the original volume for correction of the residual concentrations of NO_3, K and Ca.

All plants were initially suspended in their respective nutrient solutions on June 21, 1942, and analyses for NO_3, K and Ca were made on July 14, 1942, at the end of the first interval of absorption of nutrient elements. Similar analyses were repeated at 6-week intervals until July 1943.

Plants grown in soil, reported in figure 6, were treated with liquid fertilizers, *i.e.*, the fertilizer salts dissolved in water, applied to the leaves instead of dry fertilizer salts to the soil. Approximately 100 ml. of 0.05 M. KNO_3 and 0.05 M. $Ca(NO_3)_2$ per plant was applied to the center, or region of new leaves, different amounts of fertilizers in various treatments adjusted by different frequencies of application.

Analytical

In addition to the analysis of the nutrient solutions for NO_3, K and Ca, plant tissues were also analyzed for the same elements.

The plants were sectioned according to techniques previously reported (**11**). The leaves and stems were segregated into groups of different chronological and physiological age, as old, mature, active, and young. The individual leaves of each group were cut at variable lengths, segregating chlorophyllous from non-chlorophyllous areas and terminal from intermediate or basal sections. The basal sections of all pineapple leaves are free from chorophyll, the length of such sections decreasing with advancing age. The tissues of the basal sections, in old and mature leaves, are tough and highly fibrous. Similar tissues in active and young leaves, of meristematic status, are tender and brittle. All other sections of the leaves contain chlorophyll, the terminal sections more than others (**11**).

For the analyses of plant tissues conducted at the intervals designated in the various figures and tables, two plants were taken from each set, sectioned, and the tissues were either ashed or their sap extracted according to the requirements for the measurements of various substances. Sap extractions were made after mixing 20 grams of the tissues with 80 ml. of water, macerating in a Waring Blendor, and extracting the liquid fraction by straining through canton flannel with the application of manual pressure. For sugar determinations, aliquots of these extracts were treated with lead acetate and subsequently deleaded with $Na_2H\ PO_4$. Similar aliquots, for the determination of nitrates, were treated with $CuSO_4$, and the excess copper removed with $Ca(OH)_2$. For determinations of potassium, calcium or magnesium, the tissues were ashed, the ash dissolved in 10 ml. of 0.5 N. HCl and then diluted to 100 ml. For total nitrogen, the tissues were processed according to the Kjeldahl method, with selenium oxychloride as catalyst.

Nitrates were determined colorimetrically with phenol disulfonic acid (**1**), potassium with nitroso-R-salt (**14**), and calcium titrimetrically with $K\ MnO_4$ after precipitation with ammonium oxalate (**1**).

Results

Weights of plants and component organs

Determination of the weights of greenhouse plants were begun 80 days after planting, and repeated at approximately 6-week intervals, *i.e.*, October 21, December 1, 1942, January 12, February 22, April 6, May 18, July 1 and August 24, 1943.

The results, in figure 1, show that plant and stem weights increased more than root weights with greater amounts of nitrate in association with equiionic concentrations of calcium in cultures A to E, or of potassium in F to J. Under similar conditions, root weights did not increase in proportion to leaf or stem weights.

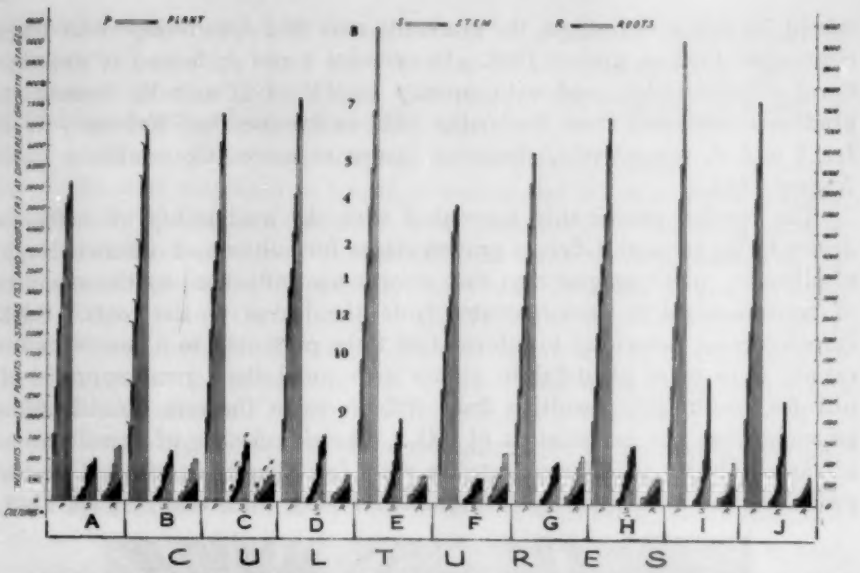

FIG. 1. Plant (P), stem (S) and root (R) weights for cultures A, B, C, D, E, F, G, H, I and J, in September (9), October (10), December (12), 1942, January (1), February (2), April (4), May (5) and July (7), 1943.

Root weights, as per cent. of total plant weights, in figure 2, showed ascending gradients at successive growth intervals from September to April and May in the low-N cultures A, F, G and B, C, or H, respectively, but descending gradients thereafter. In the high-N cultures D and E,

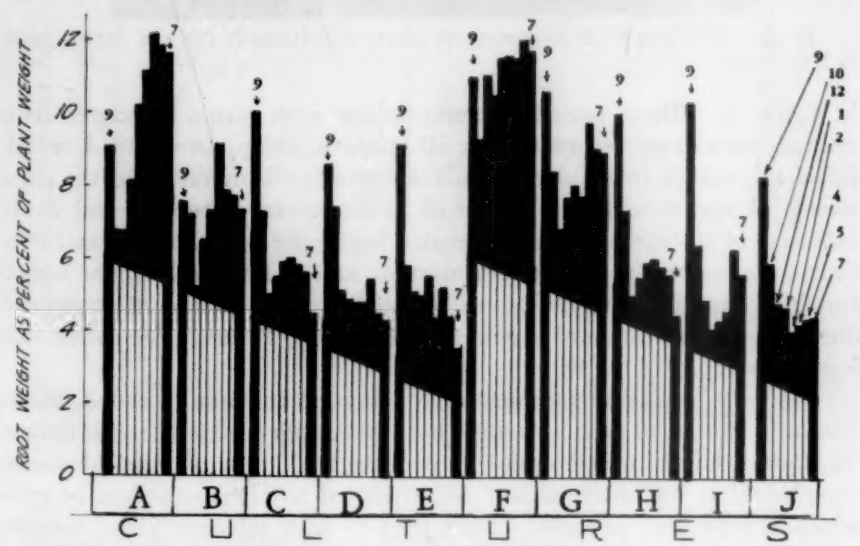

FIG. 2. Root weights as per cent. of plant weights for cultures A, B, C, D, E, F, G, H, I and J, in September (9), October (10), December (12), 1942, January (1), February (2), April (4), May (5) and July (7), 1943.

except for minor deviations, the gradients were of a descending order from September 1942 to August 1943. In cultures I and J, failing to undergo floral differentiation, and with equally high-N as D and E, descending gradients developed from September 1942 to January and February 1943 for I and J, respectively, changing thence to ascending gradients until August 1943.

The results, presumably associated with the availability of carbohydrates in the roots at different growth stages for cultures of different levels of nitrogen, might suggest that root growth was influenced by the amounts of translocationable carbohydrates from the leaves to the roots. Such carbohydrates, according to information to be presented in a future publication, were more plentiful in plants with small than great supplies of nitrates, presumably resulting from differences in the rate of utilization accompanying the assimilation of NO_3. The significance of translocation of carbohydrates at different rates to roots from leaves is reflected on the growth of the former, in the histograms for May, June and August, 1943,

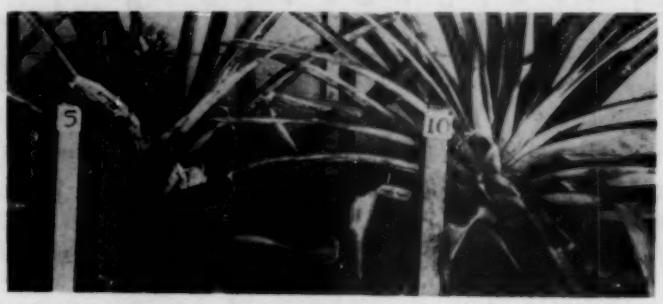

FIG. 3. Photographs of representative plants of cultures E (5) and J (10) taken in May 1943.

in figure 2. These periods, corresponding with stages of rapid fruit growth, show that root growth in all cultures, except in I and J, which failed to produce fruits, did not increase due to the diversion of the main stream of sugars to fruits instead of to roots. In cultures I and J the inflection of the curve of the histograms, beginning with January and February, the season for floral differentiation, and ending in July, the season for fruit harvest, in figure 2, was directed upward instead of downward due to the translocation of sugars to roots instead of fruits, the latter failing to develop.

Figure 3, depicting representative plants of cultures E and J, shows the latter failing to produce fruits, but leaves with brittle sclerotic tissues. The failure of cultures I and J to produce fruits but undergo abnormal morphological leaf changes may be attributed to high potassium in combination with high nitrogen, rather than to high nitrogen alone, because similar concentrations of nitrogen in combination with calcium in cultures D ad E did not interfere either with the periodicity of floral differentiations or cause abnormal morphological changes in the leaves or stem. It is

possible to assume that high concentrations of potassium, antagonizing the absorption of calcium, might have reduced the latter to deficiency levels in the tissues or interfered with its physiological functioning, with the resultant histological changes mentioned above.

In addition to the morphological changes in the leaves of cultures I and J, the stem increased in length, diameter and weight. Such changes, indicated by the ratios of stem to total plant weights for cultures with equal amounts of nitrogen but with different amounts of potassium in the nutrient solution, were, respectively, 0.088 and 0.066 for A and F; 0.095 and 0.077 for B and G; 0.104 for C and H; 0.109 and 0.174 for D and I, and 0.107 and 0.160 for E and J. Therefore, cultures A, B, I and J, with greater amounts of potassium, had higher ratios of stem to total plant weights than cultures F, G, D, and E, with less potassium.

The data suggest that different concentrations of potassium had presumably modified variously the carbohydrate-nitrogen metabolism, allowing in the cultures I and J of high potassium-high nitrogen-intermediate-calcium vegetativeness, and in cultures A, B, C, D, and E of low to high calcium-high nitrogen-intermediate potassium, floral differentiation.

Plant growth and water requirements

The curves in figure 4, depicting plant growth as measured by the

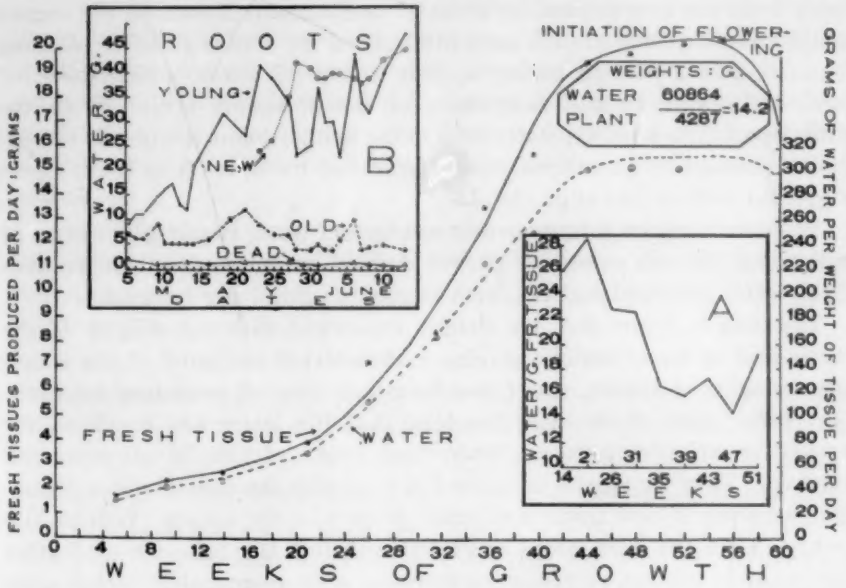

FIG. 4. Weights (grams) of fresh tissue produced per day and of water absorbed for the same period at different growth intervals for culture C, obtained as the quotient of the cumulative plant or water weights by cumulative time. 4-A. Water (ml.) required per gram of fresh tissue for the various intervals. 4-B. Water absorption by roots of different chronological and physiological ages.

weight of tissues produced per day, and water requirements by that utilized per day for different growth intervals from the time of planting to the floral differentiation period, were calculated by dividing plant and water weights by the cumulative time in days. The quotients resulting therefrom, indicative of the weight of the plant tissues produced or of the water absorbed per day, showed progressive increments with time. Results obtained previously (**10**) suggested that the growth curve of pineapple plants conforms closely to that of autocatalytic reactions.These curves, in figure 4, typical of cultures C or H, indicate that between the fifth and 52nd week after planting, the recorded mean fresh weight per plant was approximately 4287 grams and the volume of water absorbed was 60864 grams; the latter equivalent to 14.2 grams per gram of fresh tissue. Of the 14.2 grams of water, 93%, *i.e.*, $60864 - 4287 \div 60864 \times 100$, was transpired, the remainder, 7%, becoming constituent of the plant tissues.

The values reported for plant weights and volumes of water are approximations, due to the death of senile leaves and some water losses from the containers through evaporation, although proper care reduced the latter to a minimum.

In pineapple plants, where all growth except root growth emanates from meristematic regions of the apical tissues of the stem and of the basal tissues of the leaves, the increased plant growth rates with the passing of time result from the ever expanding areas of meristematic tissues in the organs mentioned. Maximal growth rates attained on the 40th week after planting culminated in a plateau lasting approximately 16 weeks, which preceded floral differentiation and completion of the ontogenic cycle. With the initiation of flowering, vegetativeness in the mother plant completely ceased, and all subsequent growth was characteristic of the developing fruit, shoots or ground suckers and slips (**10, 11**).

The data in figure 4-A, depicting amounts of water required per gram of fresh tissue for each successive growth interval, were calculated on the basis of the water absorbed and the fresh tissues produced per interval.

The data in figure 4-A are strictly concerned with the weights of the tissues and of water utilized during each interval exclusive of the cumulative weights of tissues, water and length of time of preceding intervals. They differ from those in figure 4 in that the latter are based on the cumulative weights of tissues, water and length of time of all preceding intervals. Thus the results in figure 4-A show that the rate of water utilization per gram of new tissue was much greater in the earlier (14th to 31st week) than in the later stages of growth and that the factors contributing thereto, although not carefully ascertained, were presumably higher rates of transpiration, due to greater tissue succulence, lower suberization of epidermal tissues and less shading from superimposed leaves—conditions associated more often with young and small plants in the early growth stages than with mature and large plants in the late stages.

Figure 4-B, depicting water absorption by individual roots of different chronological and physiological ages, obtained by a method reported previously (**7**), shows that young roots absorbed more water from nutrient solutions than those of more advanced chronological and physiological status. Young roots exposed a much greater surface area of meristematic, non-lignified, white tissues at the tip than old roots; similar tissue areas in much older roots were considerably smaller. Water absorption by dead roots, although extremely small, resulted presumably from inbibitional forces.

The results in figure 4-B, revealing absorption of great volumes of water by young roots, suggest that under conditions of ample water and oxygen supplies in the soil and without interference from pathogenic organisms, a small number of young roots might suffice for the partial procurement of water and mineral nutrients to the plant. Actually, under field conditions, plants having lost all the subterranean roots from pathological causes in wet areas, are often found to continue growth by the development of white, functional tips in the leaf axillary roots above the level of the soil, which absorb water and mineral nutrients applied in solution as sprays. This condition develops more often in plants with mature stem tissues, more characteristic of the post-fruiting and fruiting than pre-fruiting stages.

Absorption of nitrate and potassium

Cumulative absorption curves of NO_3 and K for the various solution cultures, in figure 5-A, showed, for the entire life of the experiment, approximately twice as great absorption of K, in grams per plant, as of NO_3. The amounts of NO_3 and K supplied to the nutrient solutions correlated positively with those absorbed by the roots with coefficients of correlation: $r = 0.95$ and $r = 0.92$ for the NO_3 and K, respectively, both statistically significant at $P = .01$.

Also of interest in the quadrangular enclosure of figure 5-A are the equivalent ratios of absorbed K to NO_3, which were, for the different cultures, as follows: A, 2.63; B, 1.43; C, or H, 0.92; D, 0.81; E, 0.79; F, 0.73; G, 0.84; I, 1.13 and J, 1.17.

The results show that in cultures A and B, with low Ca and NO_3 but with intermediate K, the ratios of absorbed K to NO_3 were higher than 1.0. Likewise, in cultures I and J, with high K and NO_3 but with intermediate Ca, ratios of K to NO_3 greater than 1.0 were obtained. In all other cultures similar ratios were lower than 1.0. The mean ratio of K to NO_3 for the combined cultures C or H, D, E, F, and G was 0.82 ± 0.10, suggesting that cations other than K, presumably Ca and Mg, were absorbed, approximately to an extent of $18 \pm 10\%$ of the cations required to equalize the negative electrostatic charges of NO_3 in excess of K, assuming that cations and anions were absorbed at equivalent amounts.

The data, for the cultures A, B, I and J, with K to NO_3 ratios greater

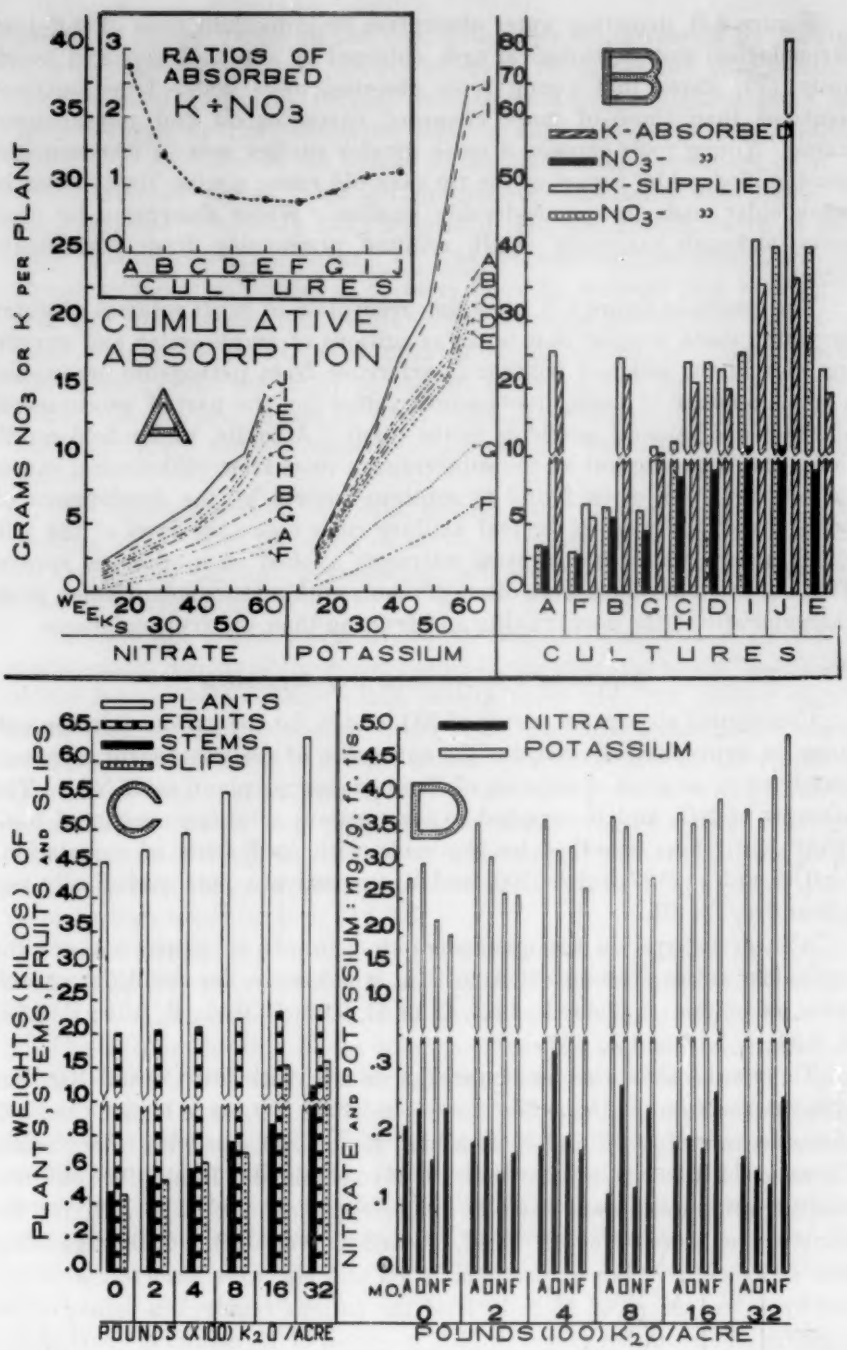

FIG. 5. A. Cumulative absorption curves of nitrate and potassium for cultures A, B, C, D, E, F, G, H, I and J. Above, in quadrangle, equiionic ratios of absorbed K to NO_3 for the same cultures. B. Weights of supplied and absorbed K and NO_3 per plant

than 1.0, suggest that anions other than NO_3 were absorbed to equalize the positive electrostatic charges of the cations absorbed in excess of NO_3.

In figure 5-B, the histograms depicting total amounts of NO_3 and K, supplied to and absorbed by the plants, reveal that potassium absorption as per cent of total supply for the various cultures was as follows: A, 87; B, 89; C, or H, 89; D, 87; E, 85; F, 87; G, 90; I, 80; J, 42. In the same figure, the columns for nitrate indicate that absorption of NO_3 as per cent of total supply for the various cultures was as follows: A, 99; B, 89; C or H, 67; D, 37; E, 21; F, 94; G, 76; I, 44 and J, 26. The results show that potassium absorption was 87 $\pm$ 3% of the total supply in all cultures except J. Similar data for nitrate show that in cultures A, B, F, G, with low nitrate content, per cent, absorption ranged from 76 to 99 of total supply. In cultures D, E, I, and J, with high nitrate content, similar values ranged from 21 to 44% of total supply.

The results show that pineapple roots absorbed more K than NO_3, suggesting differential rates for cations and anions. It is possible that the negative electrostatic charges of the root tissues might be responsible for greater attraction of K than of NO_3.

In figure 5-C, results from field-grown plants with different levels of potassium but with equal nitrogen show that high potassium applications increased plant weights progressively. Also, the ratios of stem to plant weights increased with greater amounts of added potassium as K_2O per acre, as follows: 0.1035, at zero pounds, 0.1215, at 200 pounds; 0.1230 at 400 pounds; 0.1400 at 800 pounds; 0.1450 at 1600 pounds and 0.1700 at 3200 pounds. Stem and plant weights correlated with a coefficient $r = 0.96$, statistically significant at $P = 01$. Likewise, fruit and plant weights correlated with a coefficient $r = 0.98$, statistically significant at $P = .01$.

According to the data in figure 5-C, total slip weights per plant, *i.e.*, the product of mean weight multiplied by the number of slips, increased in the same order as the amounts of potassium supplied to the plants.

The data in figure 5-B, depicting K and NO_3 levels in the basal meristematic tissues of the active D leaves (9) of the plants reported in figure 5-C, for the growth intervals of August (A), October (O), November (N) and February (F), showed descending gradients in the K concentrations for the treatments of 0, 200, 400 and 800, but ascending gradients for those of 1600 and 3200 pounds of K_2O per acre.

The K content per plant in grams calculated from the product of the plant weights, in figure 5-C, by the potassium content in the tissues of the plants harvested in February (F), in figure 5-D, was for the various treatments of K_2O per acre, as follows: 8.8 at 0 pounds; 12.0 at 200; 12.9 at 400; 15.6 at 800; 24.5 at 1600 and 31.2 at 3200.

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from June 1942 to July 1943 for cultures A–J. C. Weights of plants, fruits, stems and slips of cultures grown in the field with 500 lbs. of N and K_2O, 0, 200, 400, 800, 1600 and 3200 lbs. per acre. D. Nitrate and K content of the tissues of the cultures, in 5-C, in August (A), October (O), November (N) and February (F).

These values show that the K content per plant increased in all treatments over the zero pounds at K_2O treatment in the following multiples: at zero pounds, 1.0; at 200, 1.48; at 400, 1.49; at 800, 1.77; at 1600, 2.78 and at 3200, 3.59. The difference of K increase per plant between the treatments 200 and 400 pounds was insignificant.

Nitrates, restricted before assimilation to the non-chlorophyllous tissues of the plant (**9**) and after assimilation dispersed in organic combinations throughout the entire plant, cannot be made a basis for calculation of their concentration per plant.

Translocation of nitrate

Nitrate undergoes little or no change in pineapple roots, in the stem and in the non-chlorophyllous basal tissues of the leaves (**12, 16**), but in the chlorophyllous tissues it is converted to protein by means of intermediary reactions which have not as yet been experimentally elucidated. Ammonia, contrasted to nitrate, is assimilated in the roots, stem and non-chlorophyllous or chlorophyllous leaf tissues (**12, 16**).

The difference between nitrate and ammonium assimilation is in the reduction of the former to the latter, according to certain investigators, presumably via nitrite → hydroxylamine → ammonia. This process, though chemically possible, cannot be accepted without reservations, due to the meager quantities of nitrite and hydroxylamine concentrations as evidence for its support. Also, the quantities of amide and amino acid compounds which, with ammonium assimilation are plentiful in the tissues, are rather scarce with nitrate assimilation. Acceptance of the theory of nitrate reduction to ammonia without the high concentrations of amide and amino nitrogen compounds should of necessity presuppose the existence of a hypothetical mechanism which, with nitrate assimilation, would operate more rapidly for the converting of amides and amino acids resulting from ammonia via nitrate reduction into more complex compounds, such as proteins. Experimental information on hand is inconclusive as to the existence and operation of such mechanisms.

Figure 6, depicting the course of nitrate translocation after absorption from the roots to the stem and the leaves, and the conditions contributing thereto, reveals that nitrate concentrations in the tissues are affected profoundly by those of the nutrient solution, absorption area of root tissues, rate of plant tissue expansion by growth, light, potassium and other cations.

The nitrate content in the basal (1), transitional (2), and low chlorophyllous (3) sections of the leaves and in the cortex and stele of the stem for the plant areas old (B), mature (C), active (D), new (E) and peduncle (G), in figure 6-A, shows that it was higher in the cultures supplied with great than small amounts of nitrogen. The rapid decline of NO_3 from the basal (1) to the low chlorophyllous (3) sections resulted from its assimilation in the chlorophyllous tissues in the latter.

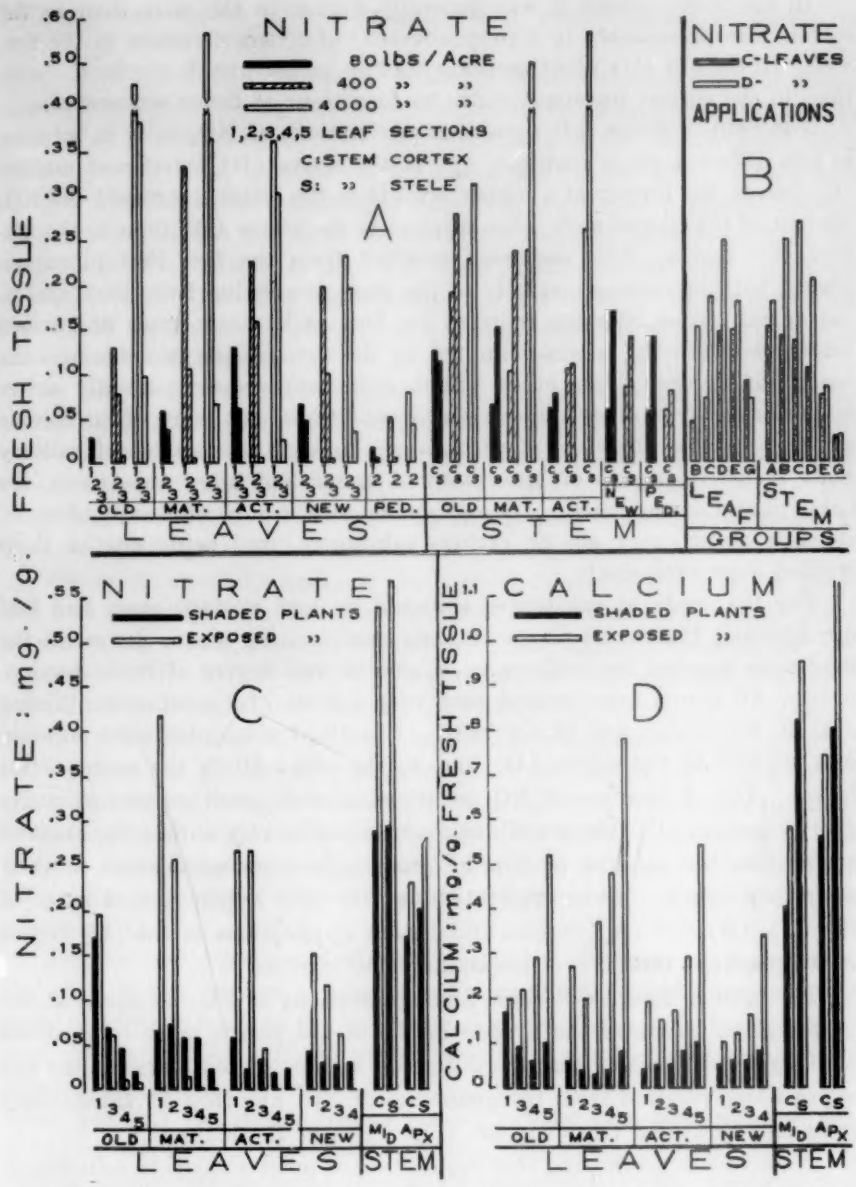

FIG. 6. A. Nitrate in different leaf and stem sections of plants supplied with 80, 400 and 4000 lbs. of nitrogen as $(NH_4)_2SO_4$ per acre. B. Nitrate in the basal leaf and apical stem sections of plants with KNO_3 and $Ca(NO_3)_2$ solutions applied to the active (D) or mature (C) leaves. C. Nitrate in different leaf and stem sections of shaded from and exposed to sunlight plants. D. Calcium in plants shaded from or exposed to sunlight.

In the stem, nitrate-N was generally higher in the stele than in the cortex, due presumably to a preponderance of xylem elements in the former. In the old (B) plant sections, similar values were lower in the stele than in the cortex, presumably due to conditions of tissue senescence.

The data in figure 6-B show that applications of NO_3 salts in solution at two different plant positions, *i.e.*, at the active (D) leaves and mature (C) leaves, the former at a higher level than the latter, increased the NO_3 content of the tissues more when applied to the active (D) than to the mature (C) leaves. This condition resulted from the fact that pineapple plants, with increasing maturity of the stem proceeding from the basal to the apical tissues, develop roots at the leaf axils which grow at various lengths between the spaces delimited by the stem on the one side and the basal leaf tissues on the other. Such roots may remain partially active under drought or other undetermined conditions, but more often become active by the development of white tips. In addition to the leaf axillary roots as active organs of absorption of water and other substances, the basal tissues of the active (D) and young (E) groups of leaves, of meristematic status, may absorb various substances, iron being one of those studied most extensively.

For the study of substances absorbed by leaf axillary roots and leaf meristematic tissues, 20 plants of early post-fruiting status grown in the field were selected for uniformity of growth and degree of fruit development. All plants were treated, each with 100 ml. of a solution containing 0.05 M. KNO_3 and 0.05 M. $Ca(NO_3)_2$. To 10 of these plants the solution was applied to the active (D) and to the other 10 to the mature (C) leaves. The application of NO_3 solutions to such plant regions as active (D) or mature (C) leaves was not confined exclusively within the areas of application but allowed to flow by gravity, wetting more areas in their downward course. Thus applications at the (C) region wetted areas of the (C), (B) and (A) regions and similar applications at the (D) region wetted areas of the (D), (C), (B) and (A) regions.

The data in figure 6-B show that applications of NO_3 solutions at the (D) regions increased the nitrate intake of the plants more than at the (C) regions, indicating that the difference in NO_3 content between the two applications resulted from the quantities of NO_3 absorbed by the axillary roots and basal leaf tissues in the (D) region of the plant.

These results, indicating that application of nutrient salts in solutions at concentrations compatible with the tolerance of the plant tissues offered a more satisfactory way of applying fertilizers than by placing the dry salts in the soil or at the basal leaves of the plant, were made the basis for the introduction, in 1932, of a method of liquid fertilizer applications which has been in use by plantations since 1937.

Conditions favoring the assimilation of nitrates, as measured by the rate of disappearance from the chlorophyllous tissues, are light, presum-

ably via combination with carbohydrates or photochemical reduction, as suggested by BURSTRÖM (**2**).

Such effects of light on nitrate intake, in figure 6-C, show that the plants exposed to light contained greater concentrations of NO_3 than the shaded plants, as indicated by the NO_3 concentrations in the non-chlorophyllous tissues in the basal sections of the leaves and in the stem. The plants shaded with black cloth contained more NO_3 per gram in the chlorophyllous sections, Nos. 4 and 5, of the mature, active and new leaves, than the exposed plants, indicating that NO_3 assimilation was inhibited in the absence of light. The NO_3 concentrations of the old (B) leaves in the exposed and shaded plants showed very small difference, presumably due to protoplasmic senescence.

In figure 6-D, calcium intake was greater in the exposed than in shaded plants, being in the same order of intake as nitrate in figure 6-C. Calcium concentrations increased in the chlorophyllous and non-chlorophyllous tissues, whereas nitrate, due to rapid disappearance by assimilation in the chlorophyllous sections, increased only in the non-chlorophyllous tissues at the basal leaf sections.

The occurrence of nitrate in the cortical tissues of the stem in figure 6-A and figure 6-C is due to the fact that in Ananas, as in many monocotyledonous plants, cortical tissues with the phloem elements as contrasted to stelic tissues with xylem elements, are not separate, but intermixed. The fibrovascular bundles in endogenous stems, as in Ananas, starting from the base of the leaf to which they respectively belong, curve inward more or less strongly towards the center of the stem, and thence gradually outward as they descend until they reach the cortex, in which the attenuated lower extremity mostly terminates. Consequently, the bundles from different heights in the stem cross in their course and it is partly owing to these connections that the cortex is not separable from the stele (**3**).

Nitrates and other nutrient elements and water after discharge from the roots at the regions of contact with the fibrovascular bundles of the stem, are translocated to the basal tissues of the leaves. In the latter tissues, lacking chlorophyll, the concentrations of nitrates compare in magnitude with those in the stem, suggesting that no nitrate assimilation had taken place in the stem. Nitrate concentrations decrease almost to zero in the chlorophyllous tissues of the leaves where they are assimilated, the rate of disappearance being inversely proportional to the concentration.

The data in figure 7, depicting nitrate concentrations in the basal (1), intermediate or transitional (2), and low-chlorophyllous (3), sections of the active (D) leaves and in the medial, apical and peduncle sections of the stem for all cultures, A to J, showed variations for the respective cultures proportional to the NO_3 concentrations of the nutrient solutions.

In the basal sections of the active (D) leaves nitrate concentrations were generally higher in all cultures at the beginning of the experiment

in September 1941, (IX), than at subsequent intervals, presumably resulting from a low rate of utilization due to small plant volumes. In the high nitrate cultures D and E, NO_3 concentrations increased after January 1942 (I), due to the high nitrate content of the nutrient solutions and to retarded plant growth following floral differentiation. In cultures A and F, with low nitrate in the nutrient solution, nitrate concentrations in the basal leaf tissues were relatively high at the beginning, becoming subsequently low and finally dropping, in May and June 1942 (V, VI), to non-measurable quantities.

In the intermediate or transitional leaf sections nitrate values were generally lower than in the basal sections, due to some assimilation by the partially chlorophyllous tissues of these sections. Except for the much lower nitrate concentrations in the intermediate than in the basal sections of the leaves at the early stages of growth, the curves at the late stages show that NO_3 concentrations were approximately the same for the basal and intermediate stem sections for the various cultures. Nitrate concentrations in the low-chlorophyllous sections (3), of the leaves were considerably lower than in the basal (1), or in the intermediate or transitional (2) sections in all cultures, due to a certain degree of assimilation by the chlorophyllous tissues. Nitrates translocated to the chlorophyllous sections of the leaves were assimilated very rapidly, as indicated by their complete disappearance under conditions of optimum light and temperature.

However, under conditions of very great nitrate supplies, *i.e.*, 4000 pounds per acre, in figure 6-A, some nitrate was found even in the terminal (5), sections of the old (B) leaves, presumably due to the lower rate of metabolic activity of the tissues of old than young leaves.

Nitrate concentrations in the stem were generally of the same magnitude as in the leaves and susceptible to comparable fluctuations.

Differences in nitrate concentrations between early and late growth intervals, *i.e.*, September (IX) and June (VI), were smaller in the apex of the stem than in the basal sections of the leaves of cultures E, D, I and J with high nitrate. In the leaves of the low nitrate cultures A and F, although NO_3 disappeared in May and June, the stem contained some NO_3 at all times in concentrations approximately proportional with the external supply.

The middle of the stem showed more fluctuations in nitrate concentrations related to the external supplies and to the rates of translocation to the leaves than other sections. For example, in cultures D, E, I and J, with high supplies of NO_3, nitrate concentrations in the middle of the stem were greater in the late, June (VI), than early, September (IX), growth intervals, presumably due to a much greater rate of NO_3 intake by the roots and deposition in the stem than of translocation from the latter to the leaves with subsequent accumulation in the stem. In cultures C or H, with moderate supplies of NO_3, the rate of nitrate intake by the roots and

translocation therefrom to the leaves *via* the stem, except for minor fluctuations, was approximately the same. In cultures A, B, F and G, with low supplies of NO_3, nitrate concentrations in the middle of the stem were consistently lower at the late than in the early growth intervals, presum-

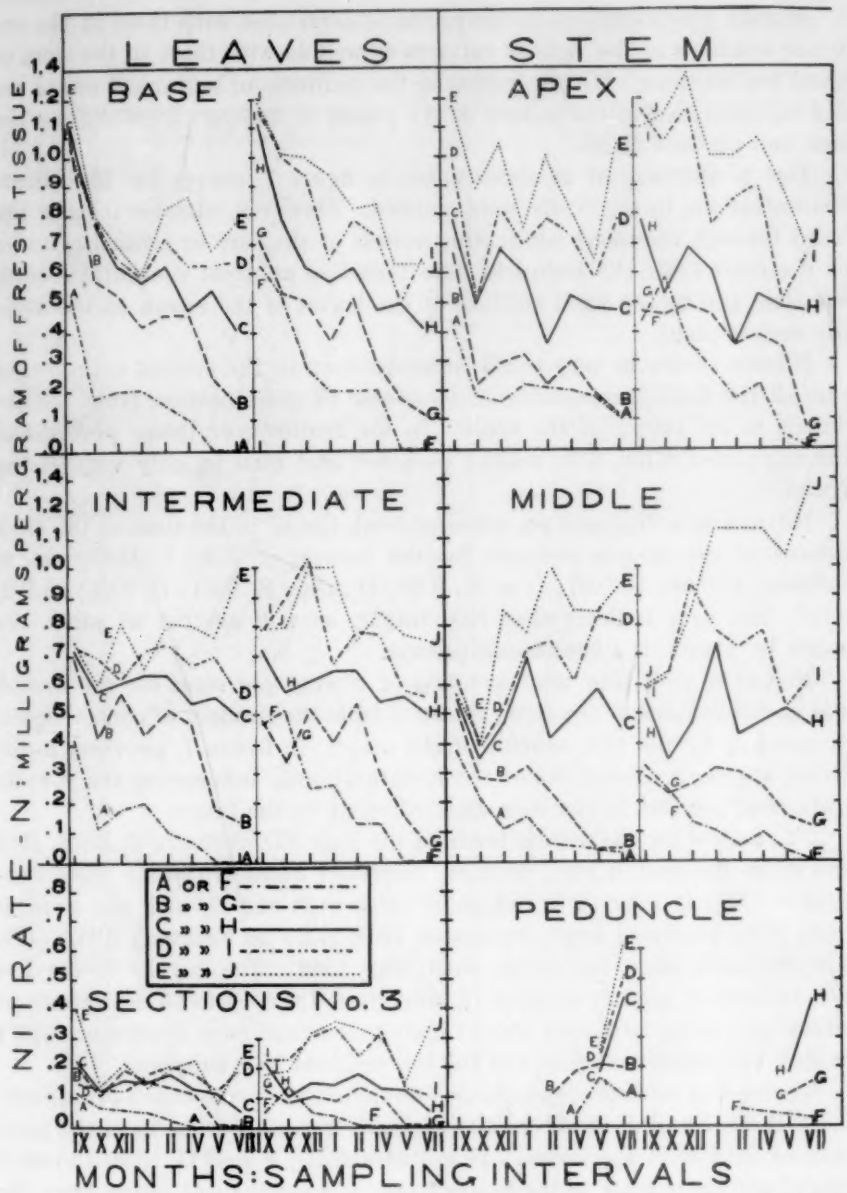

FIG. 7. Curves for the NO_3 content of the leaf and stem sections, as specified, for cultures A to J, in September (IX), October (X), December (XII), 1942, January (I), February (II), April (IV), May (V) and July (VII), 1943.

ably due to a much greater rate of translocation to the leaves from the stem than of intake by the roots and accumulation in the stem. These comparisons show how different external nitrate concentrations may affect the levels of the nitrate concentrations in different organs of the plant or sections of the same.

Nitrate concentrations in the peduncle correlated with those in the nutrient solutions of the various cultures as well as with those in the stem or basal leaf sections. Nitrate values in the peduncle of cultures I and J are not reported, due to the failure of the plants to undergo floral differentiation and produce fruits.

Due to shortage of available space in figure 7, curves for the nitrate concentrations in the fruits were omitted. However, nitrates moving upward through the water conducting vessels of the core or central cylinder of the fruit from the peduncle were found in as great concentrations in the stem and in the basal sections of the leaves of the crown or top as in the mother plant.

Nitrate occurs in very small concentrations in the central cylinder, or core of the fruit, presumably in its course of translocation from the peduncle to the crown of the fruit. In the fruitlets, or fleshy portions of the compound fruit, NO_3 seldom occurred and then in only very young fruits.

Nitrate, as milligrams per gram of fresh tissue, in the stem of the fruit crowns of the various cultures for the harvest of July 1, 1943, was as follows: A, 0.01; B, 0.07; C or H, 0.33; D, 0.45; E, 0.63; F, 0.01; and G, 0.12. The data indicate that NO_3 intake, as well as that of other elements by plants, is a continuous process.

Figure 8, depicting total amounts of nitrate per stem for the medial and apical sections of the stem, obtained from the product of stem weights, in figure 1, by the NO_3 content of the plants, in figure 7, provides a different approach of analysis of the factors directly influencing the concentrations of nitrates in the stem and indirectly in the leaves.

The curves for the nitrate levels of the high NO_3 cultures C, D, E, H, I and J, in the medial stem sections, increased uninterruptedly from September (IX) to May (V). Those of cultures B and G, with low to moderate NO_3, increased from September 1941 (IX) to February 1942 (II), but decreased from the latter until May 1942. The curves of the low NO_3 cultures A and F, ranging in magnitude from 10 to 25 milligrams of nitrate per stem, remained approximately constant from September (IX) to May (V), presumably due to the low residual NO_3 supplies.

The results indicate that nitrates accumulated in the stems of cultures C, D, E, H, I and J, as the result of greater rates of inflow from the roots than of outflow to the leaves. In cultures A, B, F and G, with a greater rate of nitrate outflow to the leaves from the stem than of inflow from the roots to the stem, the nitrate curves either remained at the same level or increased slightly in the early stages of growth with plant volumes ex-

panding at lower rates than of nitrate concentrations accruing from NO_3 intake. Similar comparisons of the nitrate curves in the apical sections of the stem showed constantly ascending gradients for cultures C, D, E, G, H, I and J, but descending gradients for A, B and F from February (II) to May (V), 1942.

Comparison of the nitrate curves for the medial and apical sections of

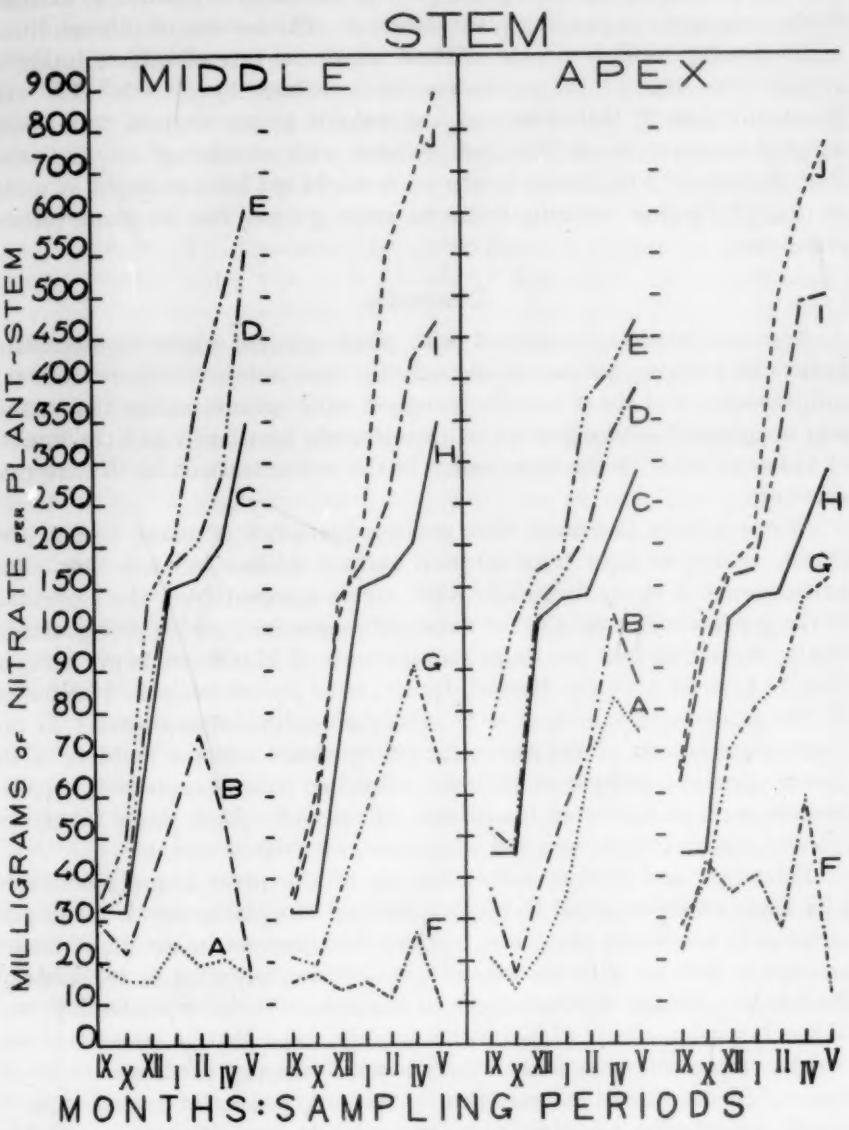

FIG. 8. Curves for the total NO_3 per stem for cultures A to J, in September (IX), October (X), December (XII), 1942, January (I), February (II), April (IV) and May (V), 1943.

the stem revealed that in the high nitrate cultures C, D, E, H, I and J, NO_3 concentrations were higher in the former than latter sections, indicating lower rates of NO_3 translocation to the leaves from the stem than from the roots to the stem. In the low nitrate cultures A, B, F and G, the nitrate level with respect to stem sections was reversed. Therefore, the gradually declining concentrations of nitrate in the basal leaf tissues, with the passing of time and plant growth, should be attributed to dilution by the constantly expanding plant volumes. The reverse of this condition might develop in high nitrate cultures where the rate of nitrate intake is greater than that of the expanding plant volume by growth. The evidence, in figure 2, that stem and leaf weights as per cent. of total plant weights increased more than root weights with advancing age, indicates that the rate of NO_3 intake by the roots might not have changed as much as that of dilution resulting from the much greater rate of plant volume expansion.

Discussion

The various data, concerned with plant growth, water requirements, uptake of nitrates, potassium and calcium from solution cultures and the soil, revealed that plant weights increased with optimal rather than maximal or minimal concentrations of the elements mentioned and the intakes of the same were in the same order as the concentrations in the external substrata.

Water uptake increased with greater quantities of plant tissues produced. Relevant data from solution culture studies indicate that pineapple plants of mean weights of 4287 grams attained from the beginning of the experiment to the time of floral differentiation, used 60,860 grams of water, suggesting that the water requirements of 17,000 plants per acre, as most frequently grown in Hawaii, for the same period would be 11.4 inches of water per square inch of soil. This value, limited exclusively to the water requirements of the plants for transpiration and the building of its tissues, does not include water losses resulting from evaporation, percolation through, or flow over the surface of the soil. Such water losses, dependent upon climatic and soil conditions, are highly variable.

Potassium and nitrate concentrations in the plant tissues correlated with those of the external nutrient solutions. In plants grown in the soil under field conditions the concentrations of potassium in the plant tissues correlated likewise with the amounts in solution supplied to the axils of the leaves. Nitrate concentrations in the tissues varied considerably, due to the favorable effects of potassium supply and light on nitrate uptake.

The various findings, with respect to application of fertilizers to plants under field conditions, suggest that in the early stages of growth, first 3-month period after planting, nitrate absorption from the soil is very low due to small plant volumes, and consequently to small areas of meristematic tissues. From the second 3-month period, NO_3 absorption increased con-

siderably, attaining maximal values during the third, fourth, and fifth periods. In the late part of the fifth and in the sixth period, NO_3 absorption decreased approximately 25 to 35% of maximum, presumably due to the cessation of growth of new leaves after the period of floral differentiation. However, NO_3 absorption ceased at no time, but remained, even after floral differentiation, approximately 65% as high as at its maximal period of absorption, *i.e.*, in the fourth and fifth periods.

Unpublished data on the absorption of other mineral elements by pineapple plants show results similar to NO_3.

The factors associated with nitrate absorption, translocation and assimilation were discussed in previous publications (**9, 12, 13, 15, 16**). Recent studies by other investigators (**2, 5, 6, 17, 19**) also summarized, in a recent review of McKee (**4**) and also by Street (**18**), are in agreement with the views expressed on nitrate assimilation in earlier findings (**8**).

Burström (**2**), discussing the assimilation of nitrate in green phototropic organs, states that it is intimately dependent upon light, and is coupled with the assimilation of CO_2. Also that no utilization of nitrate takes place without supply of CO_2 even in light, and stored carbohydrates cannot be utilized for the assimilation of nitrate, as the source of energy must be light and not respiration. Moreover, during the simultaneous assimilation, in light, of nitrate and CO_2, the latter delivers the C-compounds of which protein is built. The postulations of Burström that light is essential for the assimilation of nitrate are in satisfactory agreement with earlier results of the senior author (**8**) and those in figure 6. Warburg and Negelein (**20**) observed that light accelerated the reduction of nitrate in *Chlorella pyrenoidosa*. Also, the findings of Street, Kenyon and Watson (**17**) that nitrate assimilation was greater in the shoots of potato sprouts than in the roots is in support of nitrate assimilation in chlorophyllous tissues.

The various mechanisms of nitrate assimilation, suggested by different workers, lack undisputable experimental verification. For instance, in the nitrate reduction theory, the yields of nitrite, or hydroxylamine, the precursors of ammonia are extremely small and their identity questionable. Rautanen's (**6**) evidence, his table I, of nitrite ranging between 0.0003–0.0019 mg. and ammonia 0.062–0.194 mg. from 0 to 10 hours, respectively, and without even traces of hydroxylamine, or other related products, although interesting, is not thoroughly convincing due to the extremely small amounts of nitrite and the chances for great variability in ammonia concentrations at different diurnal intervals, growth conditions and metabolic rates.

The theory of nitrate assimilation advanced by Burström (**2**) whereby both nitrate and carbon dioxide are simultaneously assimilated in light, with the latter delivering the C-compounds for protein synthesis, deserves serious consideration and study. However, other routes must be explored for the assimilation of nitrates in addition to the one often quoted for

nitrate reduction: $NO_3 - NO_2 - NH_2OH - NH_3$, in order that the quantity and quality of the products of metabolism produced by plants with nitrate or ammonium nutrition are in agreement with those postulated by theory.

Summary

The results from experiments on the growth and chemical composition of the tissues of pineapple plants in nutrient solutions and soil cultures with different amounts of nitrogen, potassium and calcium, at 6-week intervals, yielded information as follows:

1. Increasing nitrogen in association with potassium or calcium increased the weights of the leaves and stem. Potassium increased stem weights more than calcium. Root growth, especially length rather than number of roots, increased more with low than with high nitrogen. Also, root weights as per cent. of total plant weights were lower in high than in low nitrogen cultures. Root weights were lower in cultures with plants in the fruiting than in non-fruiting stage.

2. Plants supplied with high nitrogen and calcium produced fruits at the regular season in comparison with plants of high nitrogen and potassium. Many plants with high NO_3 and K, failing to undergo floral differentiation, continued in the vegetative stage from three to six months thereafter. Such plants produced longer and thicker stems, but shorter and sclerotic leaves than similar plants with fruits.

3. Young roots with long and thick white tips absorbed more water than old roots of much greater length with highly suberized cortical tissues but with short and small diameter white tips.

4. Water requirements per plant per day were directly proportional to the quantities of newly formed tissues. However, plants utilized more externally supplied water per unit of new tissue in the early than late growth stages. The lower water requirements in the latter, due presumably to the availability of water from old plant tissues which, according to unpublished data, supplied the difference between early and late growth stages.

5. Potassium and nitrate intake by plants correlated with the concentrations of same in the nutrient solution. Equiionic ratios of potassium to nitrate absorbed by the different cultures ranged from 2.63 to 0.73. High calcium concentrations interfered less with the absorption of potassium than high potassium concentrations with calcium.

6. Nitrate concentrations in the stem and in the non-chlorophyllous basal tissues of the leaves increased in proportion to the concentrations in the substratum. Nitrate concentrations in the nonchlorophyllous tissues were increased or decreased at subsequent growth intervals by the rates of absorption by the roots and translocation to the stem and leaves, and those of assimilation by the chlorophyllous tissues of the leaves and of plant volume expansion by growth.

7. Root weights (as per cent. of total plant weights) and NO_3 concen-

trations, showing descending gradients with advancing plant age, suggest that the decline in the NO_3 concentrations might have resulted from dilution in larger volumes of aerial plant tissues due to the decreasing root to plant weight ratios.

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A STUDY OF CERTAIN NUTRIENT LEVELS FOR RASPBERRIES GROWN IN WATER CULTURES[1]

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(WITH SIX FIGURES)

Received August 22, 1949

Introduction

Several investigators (**1, 2, 5, 10, 11, 13**) have made use of plant analysis as an aid in diagnosing the nutrient requirement of plants and as a possible means of determining plant nutrient deficiencies in the soil. It has been shown that as the level of nutrients not in ample supply is increased, other factors being constant, plant growth is promoted and internal concentration of that nutrient in the plant rises, at least in certain plant organs and at certain stages of development.

Macy (**6**) has distinguished three portions of a curve relating yield to internal nutrient concentration. The section of the curve denoting "minimum percentage" shows a small increase in yield with constant internal concentration; the section of "poverty adjustment" shows an increase in yield and internal concentration; and finally the section of "luxury consumption" shows that the yield remains constant with increasing internal concentration. Macy defines the transition between poverty adjustment and luxury consumption as the "critical percentage."

Ulrich (**13**) defines the critical nutrient level of a plant as "that narrow range of nutrient concentration in which the growth rate or yield of the plant first begins to decrease in comparison to plants at a higher nutrient level." This is determined by growing plants in media supplying two different amounts of the limiting nutrient, while other growth factors are held constant. At first there is no apparent difference in growth, but later the growth of the plants in the medium with the smaller amount of the nutrient under study is less than the growth of the plants in the medium with the larger amount of nutrient. The concentration of the nutrient under study in a suitable part of the plant harvested at this point of decreasing growth is a critical concentration or nutrient level. Although the "critical nutrient level" at this point varies for different species, it tends to be relatively constant for specific organs of the same plant, provided seasonal factors of light and temperature, and concentration of other ions in the nutrient medium do not inhibit normal plant growth.

The red raspberry, *Rubus idaeus L.*, is one of the important brambles grown in the state of Washington. Although the exact fertilizer requirements for this crop are not known, the use of mixed fertilizers is a general practice considered necessary for the production of high yields. The

[1] Published as Scientific Paper No. 861, Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

objectives of the work presented here were: (1) to develop controlled nutrient deficiency symptoms in raspberry plants grown in nutrient solutions of varying levels; (2) to determine what part of the leaf tissue is an adequately sensitive indicator of the nutrient status of the plant; (3) to establish tentative critical nutrient levels of nitrogen, phosphorus, potassium, and calcium for raspberries with the object of using leaf analysis as a possible guide for determining nutrient deficiencies in the soil.

Materials and methods

Growing of plants

Red raspberry plants of the Washington variety were grown outdoors in aerated nutrient solutions by the following technique. Approximately 350 root cuttings were planted in a sand bed on April 21, 1947. Since an insufficient number of the root cuttings produced strong plants it was necessary to supplement these plants by "suckers" obtained on July 12 from the field at the Western Washington Experiment Station. The plants

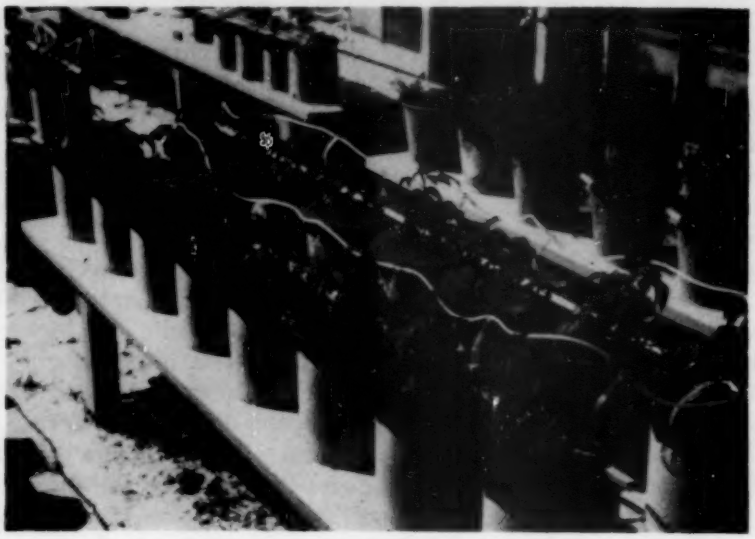

Fig. 1. Water culture set-up for nutrient level study of red raspberries.

propagated by root cuttings and the "suckers" were conditioned as follows:

The plants were placed for one day in tap water adjusted to pH 5 and then for four days in one-tenth strength Hoagland and Arnon (**3**) Nutrient Solution 1 adjusted to pH 5.5 with sulphuric acid. This solution was renewed daily. Finally the plants were placed for two to seven days in one-fourth strength Hoagland and Arnon Nutrient Solution 1 adjusted to pH 5.5. This solution also was renewed daily.

The raspberry plants used for the differential nitrogen, phosphorus, and potassium series and those for the calcium series were transferred to the standard nutrient solutions on July 19 and July 26, 1947 respectively.

Each plant was placed in a three-gallon earthenware crock containing 10 liters of nutrient solution. The stem of the plant was fixed with cotton through a hole in a paraffin-treated cork stopper which was placed in a hole in the center of a 5-ply marine plywood board covering the crock. The plywood boards were covered with two coats of W. P. Fuller Paint Co. Gilacoat paint to prevent the dissolution of soluble material in the wood and the growth of algae and fungi. Each earthenware crock contained an aerator made from porous carbon pipe attached to lucite tubing. Air carried to the aerators by Tygon tubing under a pressure of five to eight pounds per square inch escaped through the porous carbon pipes, providing continuous aeration. The system is illustrated in figure 1.

The nitrogen, phosphorus, potassium, and calcium nutrient solution series contained four levels of the element under study and each level was replicated four times. This required 16 earthenware crocks for each series. The crocks were placed on wooden benches outside the greenhouse. Distilled water and salts of analytical reagent quality were used to prepare the nutrient solutions. The chemical composition of these solutions is given in tables I and II. The nutrient solutions were never renewed nor were additions of nutrients made during the experiment, except iron which was added every week in the form of 0.5% iron citrate solution at the rate of

TABLE I

CHEMICAL COMPOSITION OF THE NUTRIENT SOLUTIONS*

SALTS USED	NUTRIENT LEVELS IN MILLIMOLES			
	I	II	III	IV
	Nitrogen Series			
$Ca(NO_3)_2 \cdot 4H_2O$	0.25	0.50	1.00	2.00
$MgSO_4 \cdot 7H_2O$	0.50	0.50	0.50	0.50
KH_2PO_4	0.44	0.44	0.44	0.44
K_2SO_4	0.28	0.28	0.28	0.28
$CaSO_4 \cdot 2H_2O$	1.75	1.50	1.00	0.00
	Phosphorus Series			
$Ca(NO_3)_2 \cdot 4H_2O$	2.00	2.00	2.00	2.00
$MgSO_4 \cdot 7H_2O$	0.50	0.50	0.50	0.50
KH_2PO_4	0.05	0.11	0.22	0.44
K_2SO_4	0.47	0.44	0.39	0.28
	Potassium Series			
$Ca(H_2PO_4)_2 \cdot H_2O$	0.16	0.16	0.16	0.16
$Ca(NO_3)_2 \cdot 4H_2O$	2.00	2.00	2.00	2.00
KH_2PO_4	0.13	0.13	0.13	0.13
$MgSO_4 \cdot 7H_2O$	0.50	0.50	0.50	0.50
K_2SO_4	0.00	0.06	0.19	0.44
	Calcium Series			
$Ca(NO_3)_2 \cdot 4H_2O$	0.05	0.20	0.60	1.80
NH_4NO_3	1.95	1.80	1.40	0.20
$MgSO_4 \cdot 7H_2O$	0.50	0.50	0.50	0.50
KH_2PO_4	0.44	0.44	0.44	0.44
K_2SO_4	0.28	0.28	0.28	0.28

* 1.25 ml. of minor element solution described in table II was added to the nutrient solution in each crock.

TABLE II

CHEMICAL COMPOSITION OF SOLUTION SUPPLYING MINOR ELEMENTS

COMPOUND	GRAMS PER LITER
H_3BO_3	2.86
$MnSO_4 \cdot 4H_2O$	2.03
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
$H_2MoO_4 \cdot H_2O$	0.02

5 ml. per crock. The volume of the solutions was maintained daily by additions of distilled water. The nutrient solutions as prepared had a pH of 5.1 to 5.5 and were adjusted weekly to a pH range of 5.0 to 5.6.

A red spider infestation was observed a week after the initiation of the experiment. The infestation was controlled by syringing with distilled water at a pressure of 45 pounds per square inch daily for three days, and every third day for nine additional days.

The raspberry plants of any one series were grown until those in the third level of concentration of the element under study appeared to display the first stages of nutrient deficiencies as manifested by a reversal in pH drift of the nutrient solution. All the plants in the series were then harvested. Those of the two lowest levels of nutrient concentration displayed advanced degrees of the nutrient deficiency, whereas those in the fourth and highest level were normal and healthy. Deficiency symptoms had been developed previously with plants in a separate experimental test in order to become familiar with the symptoms.

PLANT SAMPLING

The raspberry plants were cut at the level of the cork stoppers and rinsed immediately in distilled water. The first six physiologically mature leaves, beginning with the third or fourth leaf from the growing tip down, were selected from each plant for chemical analysis. The petioles and leaf blades were separated and placed in sample bottles. The remaining mature and senescent leaves, the meristematic or immature leaves, and the roots and stems were placed in separate sample bottles. The petiole and leaf blade samples selected for chemical analysis and the remaining plant parts were weighed after being dried at 70° to 75° C for 12 hours. The plant tissues were wet-ashed by the method of KELLEY, *et al.* (**4**) and the ash was brought to a volume of 100 ml. (solution A). A 25-ml. aliquot of solution A was made up to a volume of 250 ml. (solution B) for nitrogen and phosphorus determinations. A 50-ml. aliquot of solution A was evaporated to dryness in a 150-ml. beaker and heated in a muffle furnace at 530° C for 20 minutes to drive off ammonia and excess sulphuric acid. The residue was taken up with four drops of concentrated hydrochloric acid and 10 ml. of water, transferred to a 50-ml. volumetric flask, and made to volume (solution C) for potassium, calcium, and magnesium determinations.

The nitrogen was determined by the method of KELLEY, *et al.* (**4**) and

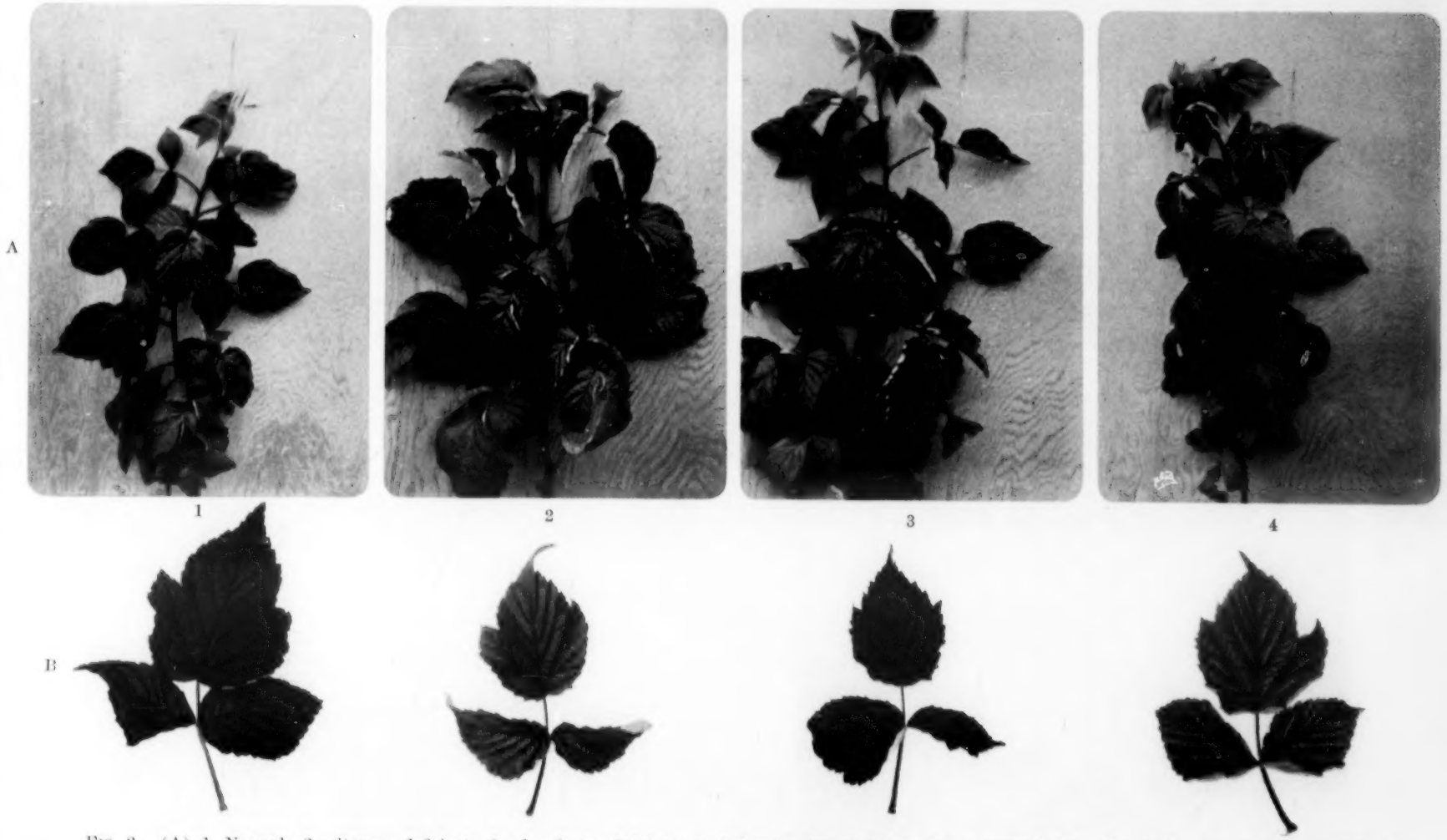

FIG. 2. (A) 1, Normal; 2, nitrogen deficient; 3, phosphorus deficient; and 4, potassium deficient plants, respectively. (B) 1, Normal; 2, nitrogen deficient; 3, phosphorus deficient; 4, potassium deficient leaves, respectively.

the phosphorus by the method of PEECH, *et al.* (**8**), slightly modified, first by neutralizing solution B with 5N NaOH to the phenolphthalein endpoint, and then adding four drops of concentrated H_2SO_4.

The potassium was determined by the method of KELLEY, *et al.* (**4**), the calcium by the method of PEECH (**7**), and the magnesium by the method of SANDELL (**9**). Aliquots of 10 ml. of solution C were evaporated to 5 ml. for the potassium and calcium determinations, and 5-ml. aliquots of solution C were used for the magnesium determinations. All the analyses were made in duplicate, except those for calcium for which single determinations were made because of shortage of ashing solution.

Experimental results

During the course of the experiment it was noted that the usual pH drift toward an alkaline reaction of the nutrient solution was generally reversed toward a more acid reaction five to eight days before noticeable foliar deficiency symptoms appeared. This reversal was not due to changes in nitrogen nutrition as only nitrate nitrogen was present except in the calcium series, but is thought to be in part the result of some change in the metabolic activity of the plant caused by the lack of the nutrient element under study. When this pH reversal was observed in the third level of concentration of the nutrient under test the plants of the entire series were harvested.

FOLIAR DEFICIENCY SYMPTOMS

Approximately 30 days after the experiment was started incipient foliar deficiency symptoms could be noted in the plants growing in the solutions containing the lowest levels of the nutrients calcium, nitrogen, and phosphorus. The following week the early stages of potassium deficiency became apparent in the plants growing in the solutions with the lowest level of potassium nutrition. In the interim, the plants in the solutions of the second levels of nutrition had developed intermediate deficiency symptoms. In some instances, however, a few of the largest plants of the second levels of nutrition had exhausted the supply of the limiting element and showed deficiency symptoms about the same time as the plants in the lowest levels of nutrition.

NITROGEN DEFICIENCY.—The beginning of nitrogen deficiency in the raspberry plants was indicated by a slightly lighter green color of the leaves, retardation of growth, and light gray color of the roots. As the deficiency of nitrogen became acute, the leaves acquired a still lighter green color, and growth virtually ceased, but advanced chlorosis was never apparent. The leaves hardened and curled or cupped inward, and the mesophyll ridges between the veins were colored with a reddish bronze pigment. The root system finally became dark gray in color. The foliar symptoms of nitrogen deficiency are illustrated in figure 2.

PHOSPHORUS DEFICIENCY.—Abnormally dark green foliage with a shiny, waxy appearance was the first symptom of phosphorus deficiency in the

TABLE III

COMPOSITION AS PERCENTAGES OF THE DRY WEIGHTS OF BLADES AND PETIOLES OF RED RASPBERRIES GROWN IN NUTRIENT SOLUTIONS WITH DIFFERENT LEVELS OF NITROGEN, PHOSPHORUS, POTASSIUM, AND CALCIUM

Nutrition series	N series		P series		K series		Ca series	
Tissue	Blades	Petioles	Blades	Petioles	Blades	Petioles	Blades	Petioles
Level of nutrient concentration	N, %	N, %	P, %	P, %	K, %	K, %	Ca, %	Ca, %
I	2.06	0.86	0.14	0.13	0.57	0.31	0.08	0.11
II	2.19	0.95	0.18	0.14	0.73	0.41	0.08	0.12
III	2.86	1.23	0.29	0.30	1.02	0.74	0.07	0.17
IV	3.65	1.62	0.35	0.39	1.47	2.97	0.17	0.25
L.S.D., .05 level	0.57	0.36	0.02	0.06	0.24	0.70	0.05	0.05
L.S.D., .01 level	0.80	0.51	0.03	0.08	0.34	0.99	0.08	0.07

raspberry plants. Progressive symptoms were manifested by reduction in leaf size, stiffening and curling of the leaves, and intensification of the dark green color which changed to a coppery purple. This is illustrated in figure 2. The older leaves became mottled, developed necrotic areas, and died. The roots became progressively darker in color until in the advanced stages of phosphorus deficiency they were a matted clump of black roots.

Potassium deficiency.—The appearance of a reddish brown pigmentation of the inter-vein mesophyll ridges of the matured leaves of intermediate age was the first symptom of potassium deficiency. As the deficiency became more acute, the older mature leaves of the raspberry plants showed a somewhat bronze coloration, and areas of dead tissue developed. Continued potassium deficiency caused the leaves to become stiffer and to curl, or roll. The tissue around the veins had a normal green color except for dead areas. The foliar symptoms of potassium deficiency are shown in figure 2.

Calcium deficiency.—The initial symptoms of calcium deficiency were manifested by a dying back of the root tips and the formation of small club-shaped swollen areas behind the dead root tips. New rootlets developed behind the dead portion so that the root system was a compact mass of short growths. The only noticeable foliar symptoms of calcium deficiency appeared as a reduction in leaf size, and hardening and curling of the leaves.

Sensitive leaf part

The analytical results are expressed as a per cent. of the element based on plant material dried for 36 hours in a vacuum oven at 75° C. The

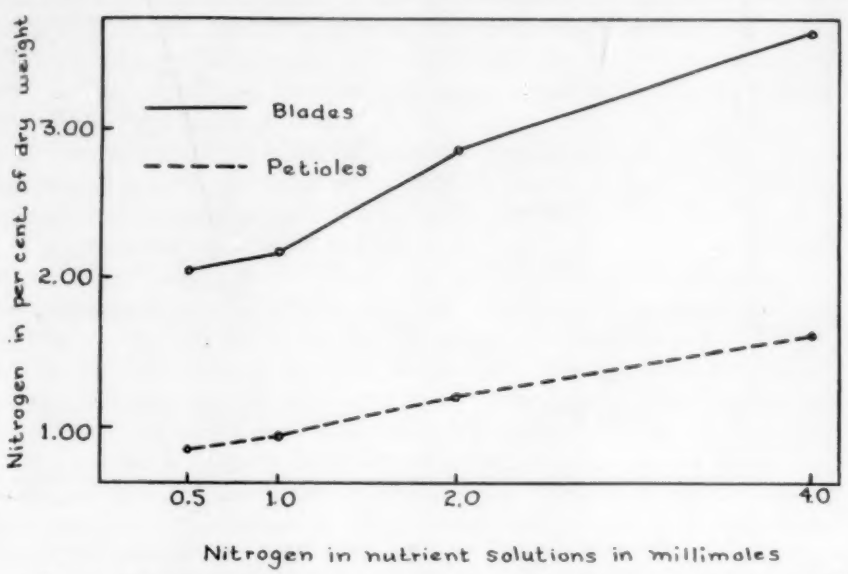

Fig. 3. Percentage nitrogen in raspberry blades and petioles as related to the initial nitrogen level of the nutrient solutions.

mean nitrogen, phosphorus, potassium, and calcium contents of the blade and petiole tissues of the four replicates in each treatment for each of the four nutrient solution series are given in table III and in figures 3 to 6. The values for nitrogen as illustrated in figure 3 show that differences in the nitrogen nutrition are much greater for the blade tissue than for the

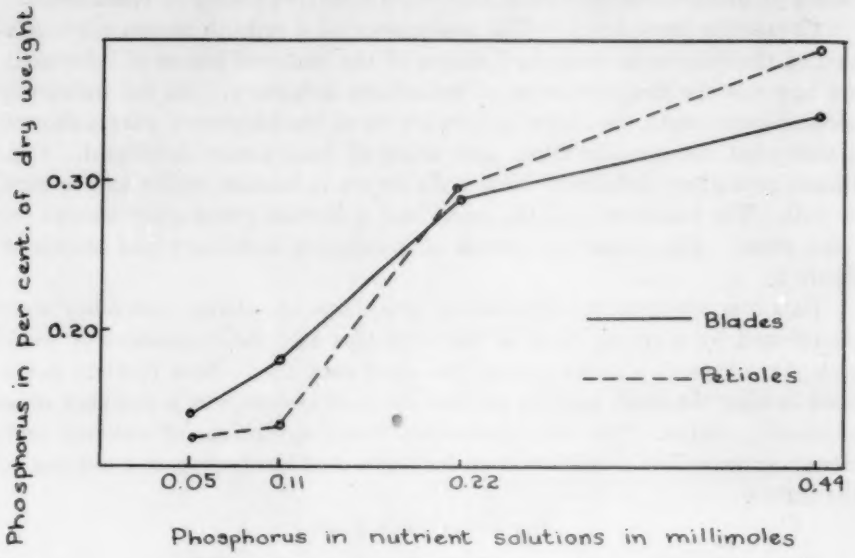

FIG. 4. Percentage phosphorus in raspberry blades and petioles as related to the initial phosphorus level of the nutrient solutions.

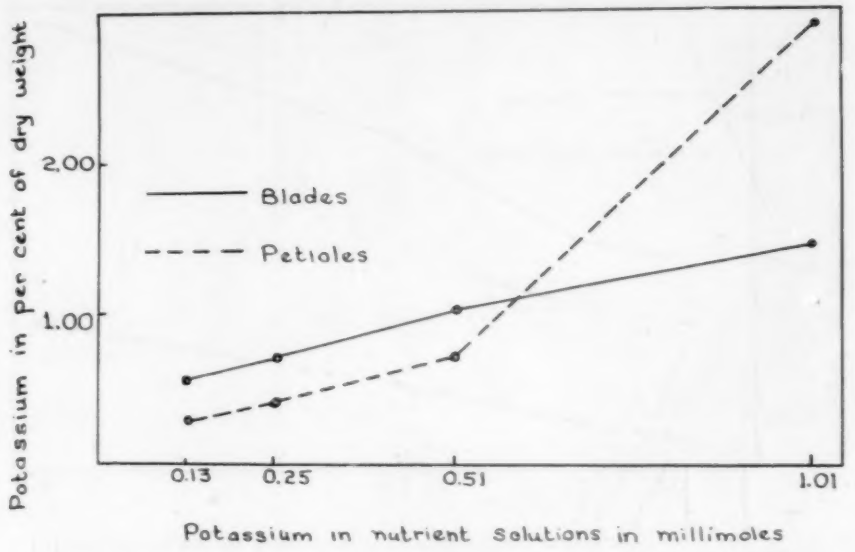

FIG. 5. Percentage potassium in raspberry blades and petioles as related to the initial potassium level in the nutrient solutions.

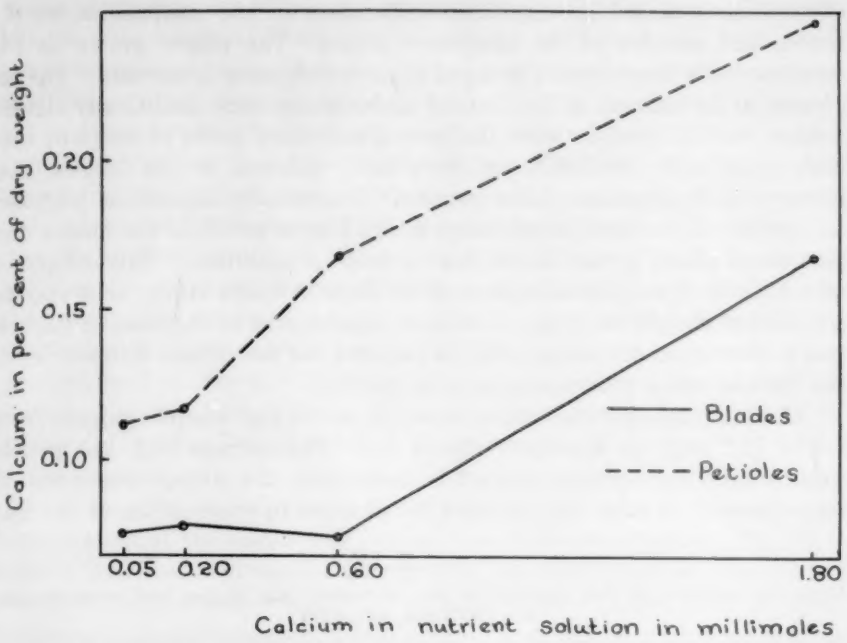

FIG. 6. Percentage calcium in raspberry blades and petioles as related to the initial calcium level of the nutrient solutions.

petioles except in the low levels of nitrogen nutrition. The blades also contained a greater percentage of nitrogen than did the petioles. The fact that the blades of mature leaves appear to be more sensitive than the petioles to changes in nitrogen concentration in the growth medium indicates that the use of blade tissue is preferable in determining the total nitrogen status of the plant.

A comparison of the phosphorus values for blades and petioles of the raspberry plants in the phosphorus series is shown in figure 4. The differences in percentage of phosphorus in the two tissues are small.

The potassium contents of the blades and petioles in relation to the potassium concentration in the nutrient solutions of the potassium series are shown in figure 5. The effect of the different levels of potassium in the nutrient solution on the potassium content of the blades and petioles was small in the first three levels. The petiole tissue shows a much greater difference in percentage of potassium in the fourth level nutrient solution where luxury consumption was probably induced.

The curves in figure 6 showing the calcium content of the blades and petioles of the raspberry plants for the calcium series show that the petioles contain a larger percentage of calcium and have a slightly greater range of calcium content than the blades.

It is noted from the data in table III that the differences in concentration of nitrogen, phosphorus, potassium, and calcium in the two low levels

of the series caused no significant differences in the composition of the blades and petioles of the raspberry plants. The plants grown in the solutions with these levels displayed distinct deficiency symptoms. The increases in the content of the element under study were statistically significant at the 5% level between the second and third levels of nutrient concentration with the following exceptions: calcium in the blades, and nitrogen and potassium in the petioles. Statistically significant increases in content of the nutrient elements studied were noted in the blades and petioles of plants grown in the fourth level of nutrition. This difference may indicate luxury consumption of the element under study, or it may be a sign that the plants of the third level of nutrition at the time of harvest had a lower nutrient content than is required for the critical nutrient level, the level at which growth begins to be limited.

The leaf blade-petiole weight ratios of the 64 leaf samples ranged from 5.7 to 10.0 with an average value of 7.8. The average leaf and petiole weights and blade-petiole ratios for each level of nutrient concentration are presented in table IV. Should the changes in composition of the leaf

TABLE IV

MEAN DRY WEIGHTS OF THE SAMPLES OF RED RASPBERRY LEAF BLADES AND PETIOLES USED FOR CHEMICAL ANALYSIS

	LEVELS OF NUTRIENT CONCENTRATION			
	I	II	III	IV
Nitrogen Series				
Weight of blades, gm.	1.40	1.42	1.70	1.78
Weight of petioles, gm.	0.20	0.20	0.21	0.23
Blade/petiole ratio, gm.	7.00	7.10	8.10	7.70
Phosphorus Series				
Weight of blades, gm.	2.06	2.22	2.28	1.55
Weight of petioles, gm.	0.24	0.29	0.27	0.21
Blade/petiole ratio, gm.	8.60	7.70	8.40	7.40
Potassium Series				
Weight of blades, gm.	1.86	2.31	2.43	2.15
Weight of petioles, gm.	0.24	0.28	0.34	0.26
Blade/petiole ratio, gm.	7.80	8.30	7.10	8.30
Calcium Series				
Weight of blades, gm.	3.19	3.61	3.24	3.04
Weight of petioles, gm.	0.38	0.49	0.46	0.44
Blade/petiole ratio, gm.	8.40	7.40	7.00	6.90

blade be less sensitive than those of the petioles to changes in nutrient concentration, the use of entire leaves would result in a dilution of one part of the sensitive petiole tissue with 7.8 parts of less sensitive blade tissue. This would decrease the accuracy of the determination of the nutrient status of the plant. Whenever possible the plant tissue that is the more sensitive indicator of the nutrient status of the plant should be used for analysis.

Critical nutrient values

Foliar deficiency symptoms displayed by plants occur after the critical

percentage defined by MACY (**6**) or the critical nutrient level of ULRICH (**13**) has been reached. This critical nutrient level marks the time when growth is beginning to be limited by the lack of the nutrient element under study.

The content of the element under study found in the blades and petioles of the plants grown in the third level of nutrition probably was close to representing the critical nutrient level under the conditions of the experiment. As noted by ULRICH (**12**) these values may be lower than those obtained under field conditions. Moreover, it was observed by VANDECAVEYE (**14**) that the raspberry leaves of bearing canes grown under field conditions contain less nitrogen, phosphorus, and potassium, and more calcium than the leaves of the non-bearing canes. The values obtained in the study presented here are for leaf tissue from non-bearing canes grown in culture solutions. On the basis of these results and for the experiments described tentative values for critical nutrient levels were established.

The critical level of total nitrogen for raspberries is approximately 2.9% in the leaf blades. Since the relative change in the total nitrogen concentration of the blades was greater than it was for petioles (fig. 3) it appears that the analysis for nitrogen in the blades rather than in the petioles is preferable as a means for estimating the nitrogen status of raspberries.

The experimental results indicate that the critical phosphorus level for raspberries is approximately 0.3% for either blades or petioles. Differences in phosphorus content of these two plant parts although slightly in favor of the petioles (fig. 4) did not vary greatly as affected by different phosphorus levels in the nutrient solutions.

According to the data (table III and fig. 5) the critical potassium level for raspberries is approximately 1.0% for the blades and 0.7% for the petioles. Use of petioles for analysis in establishing the potassium status of the plant would seem to be preferable, because the petioles had a slightly lower potassium content than the blades when there was a deficiency of potassium and a much larger content when there was an adequate supply of potassium.

The data in figure 6 do not indicate a distinct critical calcium level for raspberries. It appears to be approximately 0.2% in the petioles. Petioles contained greater amounts of calcium than blades, and for this reason they seem to be preferable for analysis in establishing critical calcium nutrient levels.

No attempt was made to determine the critical magnesium level and the analytical results for this element are not reported. The data obtained indicate that the leaf blades have a higher content of magnesium than the petioles. Since magnesium is present in plant tissue in such small quantities, it is believed to be preferable to use leaf blades for the determination of this element.

The tentative critical nutrient levels reported for nitrogen, phosphorus,

and potassium are in good agreement with the low values reported in a plant nutrient survey of 50 red raspberry fields by VANDECAVEYE (**14**).

Summary

Red raspberries of the Washington variety were grown in nutrient solutions containing four levels of nitrogen, phosphorus, potassium, and calcium. During the growing period distinct foliar deficiency symptoms were displayed by the plants in the two lowest concentration levels of each nutrient element. Descriptions and photographs of leaves and plants were presented to explain and show the foliar symptoms of nitrogen, phosphorus, potassium deficiencies in raspberry plants.

When the usual pH drift toward an alkaline reaction of the nutrient solution was reversed toward a more acid reaction, this occurrence was taken as a sign that the element under study was beginning to limit growth. All the plants of the entire series were harvested when their growth in the third concentration level of each nutrient began to be limited by lack of the nutrient element under study. Leaf samples of the first six physiologically mature leaves, beginning with the third or fourth leaf from the growing tip, were taken from each plant at harvest time. The leaf blades and petioles were analyzed separately for total nitrogen, phosphorus, potassium, calcium, and magnesium and the analytical data were used to evaluate the critical nutrient levels of raspberries for the first four elements.

The results of these studies indicate that the analysis of leaf petioles better reflected the phosphorus, potassium, and calcium status of the raspberry plant than the analysis of the leaf blades, whereas blade analysis was preferable in determining the nitrogen and magnesium status of the plant. Expressed in terms of the dry weight of plant material and under the conditions of the experiments critical levels of various nutrients were tentatively established. The value for total nitrogen in raspberry leaf blades is approximately 2.9%. The value for total phosphorus in petioles or blades is approximately 0.3%, that for total potassium in the petioles approximately 0.7%, and that for total calcium in the petioles approximately 0.2%.

This work was supported by a grant from the American Potash Institute, Incorporated. The assistance of Dr. C. D. Schwartze, Western Washington Experiment Station, in supplying the raspberry root cuttings and suckers for this study is gratefully acknowledged.

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CATALASE ACTIVITY IN CHLOROPLAST PIGMENT DEFICIENT TYPES OF CORN

H. C. Eyster

(WITH ONE FIGURE)

Received June 21, 1950

Catalase is the enzyme which releases gaseous oxygen from hydrogen peroxide and is definitely known to occur in chloroplasts. Neish (**5**) has measured the catalase activity of separated chloroplast matter and has compared it with that of whole mashed leaves. His measurements showed that all the catalase of green leaves was concentrated in the chloroplasts. However, Krossing (**4**) reported that he found catalase both in the chloroplasts and in the other parts of the cytoplasm.

Methods and materials

Catalase activity was determined by the "catalase tube" method of Knott (**3**) who measured the oxygen evolved from H_2O_2 at a constant temperature and with constant shaking for a five-minute interval, using exactly one gram of macerated tissue each time. The temperature of the water bath was 27.5° C $\pm$ 0.5, and the catalase tube was shaken at a constant frequency of 140 revolutions per minute. The motor-driven apparatus was improved by replacing the leather belt with chain and gears. This eliminated all variation due to slippage, and standardized the shaking considerably. Present too, were an ammeter and a voltmeter to measure electric DC current feeding into the motor. A rheostat was installed to regulate the current and thus have the motor maintain a constant speed of 140 rpm.

The sample was prepared by taking 1 gm of unblemished fresh leaves. These were cut with scissors, disregarding size and shape, and placed in small mortar. To this was added 1 gm U.S.P. precipitated $CaCO_3$, approximately 1 gm reagent grade sea sand 60–120 mesh, and 1–2 ml distilled water, depending upon dryness of sample. The mixture was ground to a fine paste using large pestle for about seven minutes. This was washed with distilled H_2O into a small bottle. The total volume of water added was 25 ml. This was found to be the most convenient amount for rinsing mortar and pestle.

After shaking the mixture, a 2 ml portion was quickly withdrawn by use of a large orifice pipette and transferred to one arm of a catalase tube. Five per cent hydrogen peroxide was used to which some $CaCO_3$ had been added, and the mixture was shaken. This mixture was kept stoppered and standing in ice water to keep the H_2O_2 at constant concentration by reducing decomposition to a minimum. Two milliliters of this was with-

drawn by pipette and placed into the other arm of the catalase tube. To get checks the ends did not need to be the same for sample and for H_2O_2 respectively. The charged catalase tube was carefully secured (level and in line with rod) to stopper on shaft and allowed to set in the bath exactly three minutes to come to temperature of bath. Meanwhile the water level in gas burette was adjusted and the air exit clamped. It was made certain that the catalase tube would fit well up on the stopper. The motor and stopwatch were started simultaneously. The gas burette reading was taken immediately at end of five minutes by adjusting the water level during the run.

Chlorophyll-deficient pedigrees of corn obtained from Dr. H. H. Smith of Maize Co-operation Laboratory at Cornell University were the ones used. The seeds were multiplied on a local plot on two successive summers by self-pollinating the normal-appearing plants in the albino and yellow seedling pedigrees.

Catalase activity measurements were made on albino and normal green seedlings in the same pedigree, and on yellow and normal green seedlings, also in a pedigree by themselves. There were pedigrees in which all three —albino, yellow and green seedlings—appeared and could be compared for catalase activity. Pedigrees 4810 and 4811 segregated three green : one albino seedlings in seeds from some plants, segregated three green : one yellow seedlings in seeds from other plants, and in seeds from still other plants segregated nine green : three yellow : four albino seedlings.

Results and discussion

The catalase measurements extended over a period of about three months and are presented in table I. In general, albino seedlings produced from 0 to 3 ml of O_2, yellow seedlings from 0 to 6 ml and green seedlings from 7 to 14 ml. Each value is an average of three to four close readings (usually within 0.2 ml). In the first experiments with a belt, there were some readings which had to be discarded. Usually five or six or seven readings had to be taken to get four close readings. With chain and gears in later experiments all readings were much closer, none had to be discarded and only three measurements were made.

From 16 samples constituting 64 acceptable measurements, the catalase activity of albino seedlings averaged 1.54 ml of O_2, from 14 samples constituting 52 acceptable measurements the catalase activity of yellow seedlings averaged 2.83 ml of O_2 and from 26 samples constituting 104 acceptable measurements the catalase activity of green seedlings averaged 10.14 ml of O_2. The catalase activity, within a phenotype, varied and this was most probably due to factors of seedling age, temperature of greenhouse, and light intensity during seedling growth. There was also apparently a variation from pedigree to pedigree.

The effect of illumination on catalase activity was determined. Dark-grown (etiolated) and light grown, genetically green corn seedlings were

TABLE I

MEASUREMENTS OF CATALASE ACTIVITY IN PEDIGREES OF CHLOROPHYLL-DEFICIENT CORN. CATALASE ACTIVITY IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	ALBINO SEEDLINGS	YELLOW SEEDLINGS	GREEN SEEDLINGS
		days	*ml.*	*ml.*	*ml.*
481 (2)	1/ 6/49	15	1.53 (4)*		13.71 (4)
481 (217) Wx**	1/13/49	11	1.48 (4)		13.66 (4)
481 (217) wx	1/14/49	12	1.06 (4)		12.34 (5)
482 (1)	1/17/49	12	2.89 (6)		11.56 (4)
481 (230)	1/19/49	14	1.62 (4)		9.84 (5)
483 (3)	1/26/49	16	2.00 (4)		11.12 (4)
484 (2)	1/26/49	16	.81 (4)		11.59 (4)
4810 (10)	1/31/49	14	.54 (4)		7.79 (4)
484 (9)	2/ 3/49	10	1.12 (4)		11.29 (4)
482 (1)	2/ 4/49	11	2.58 (4)		7.08 (4)
483 (8)	2/ 7/49	14	.36 (4)		10.60 (4)
4811 (14)	3/ 2/49	14	.41 (3)		9.27 (3)
	Average:		1.45 (49)		10.86 (49)
4810 (43)	1/ 3/49	12		2.35 (4)	6.90 (4)
485 (3)	1/20/49	10		2.00 (4)	11.38 (4)
485 (3)	1/24/49	14		4.66 (4)	11.98 (4)
485 (3)	1/27/49	17		3.92 (4)	11.60 (4)
486 (4)	1/28/49	9		4.80 (4)	11.70 (5)
486 (4)	2/ 2/49	14		.64 (4)	10.80 (4)
487 (3)	2/16/49	16		1.06 (4)	7.01 (4)
489 (52)	3/ 1/49	13		1.03 (3)	7.50 (3)
4811 (45)	3/15/49	13		.92 (3)	11.89 (3)
489 (89)	3/16/49	13		1.71 (3)	6.61 (3)
	Average:			2.40 (37)	9.87 (38)
4810 (5)	1/ 4/49	13	2.13 (4)	3.50 (4)	6.67 (5)
4810 (10)	1/25/49	8	2.10 (4)	5.98 (4)	9.72 (4)
4811 (various)	2/15/49	15	2.02 (4)	3.47 (4)	7.66 (4)
4811 (10)	3/11/49	13	.94 (3)	2.17 (3)	10.99 (4)
	Average:		1.86 (15)	3.89 (15)	8.64 (17)
	Grand Average:		1.54 (64)	2.83 (52)	10.14 (104)

* Parathetical value is number of trials constituting this average value.
** Wx = starchy; wx = waxy in same pedigree.

measured for catalase activity (table II). The etiolated seedlings always had a significantly higher catalase activity (figure 1). The light grown seedlings had a catalase value about ⅓ less than that of etiolated seedlings of the same age and in the same pedigree. On an average, the catalase value of light-grown ones was 68% of catalase value of etiolated seedlings. Etiolated seedlings giving a catalase value of 11.63 ml of O_2, when exposed to greenhouse light for from five to seven hours showed a reduction in catalase activity of from 24% to 30%, corresponding roughly to the difference in catalase value between etiolated and normal green seedlings.

Dark-grown albino corn seedlings, surprisingly, have a high catalase value approaching that of etiolated, genetically green corn seedlings. 481(11) albino dark-grown corn seedlings gave a catalase value as high as 10.05 ml of O_2 compared to 11.63 ml of O_2 for 481(11) genetically green

TABLE II

MEASUREMENT OF CATALASE ACTIVITY IN DARK-GROWN AND LIGHT-GROWN GENETIC GREEN CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	CATALASE ACTIVITY	
			DARK-GROWN	LIGHT-GROWN
		days	*ml.*	*ml.*
Commercial	10/14/48	14	9.02 (4)	5.28 (5)
Commercial	1/10/49	19	10.51 (4)	7.05 (5)
Commercial	1/12/49	16	9.39 (4)	7.64 (4)
		Average:	9.64 (12)	6.59 (14)
			100%	68%
481 (11)	3/10/49	15	11.63 (3)	(100%)
	Illuminated 5 hrs.		8.16	(70%)
	Illuminated 7 hrs.		8.88	(76%)

dark-grown corn seedlings. Comparative measurements were made on dark-grown and light-grown albino corn seedlings (table III). The dark-grown ones always had a high catalase activity and the light-grown ones always had a low catalase activity (fig. 1). Illumination of dark-grown albino corn seedlings showed a rapid decline in catalase value (table III).

TABLE III

MEASUREMENT OF CATALASE ACTIVITY IN DARK-GROWN AND LIGHT-GROWN ALBINO CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	CATALASE ACTIVITY	
			DARK-GROWN	LIGHT-GROWN
		days	*ml.*	*ml.*
481 (220)	2/21/49	11	7.00 (4)	1.10 (4)
			8.03 (3)	
481 (11)	3/ 9/49	14	10.05 (3)	1.64 (3)
	Illuminated 4 hrs.		7.31 (3)	
481 (11)	3/10/49	15	8.02 (3)	
	Illuminated 6 hrs.		2.33 (3)	

Hence, albino plants are capable of producing catalase in normal amounts, but in the presence of light the enzyme is subject to photo-oxidation, accounting for the low catalase activity in light-grown albino plants.

Dark-grown yellow seedlings can usually be distinguished from normal etiolated seedlings in same pedigree by a difference in the shade of color. In some pedigrees the difference is very marked, while in others there may not be any apparent difference. Whenever there is a difference, the normal, etiolated seedlings have a deeper yellow color than the dark-grown yellow ones. These dark-grown yellow seedlings registered a high catalase activity (table IV and figure 1). Presumably as with albino plants,

TABLE IV

MEASUREMENT OF CATALASE ACTIVITY IN DARK-GROWN YELLOW AND GENETICALLY GREEN CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	DARK-GROWN YELLOW	DARK-GROWN GENETIC GREEN
		days	*ml.*	*ml.*
486 (16)	8/11/49	9	11.23 (3)	13.15 (2)
486 (34)	9/14/49	12	10.28 (2)	11.72 (2)
		Average:	10.85 (5)	12.44 (4)

yellow seedlings are just as capable of forming catalase as are normal plants.

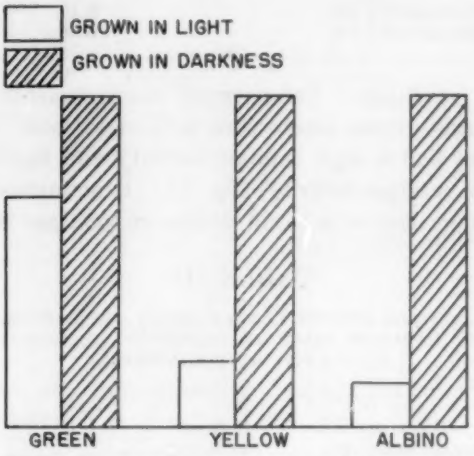

FIG. 1. Diagrammatic representation of the relative catalase activity in corn seedlings grown both under natural illumination of greenhouse and in darkness.

Table V presents further data on the effect of light on catalase activity in corn seedlings. The experiments were performed under artificial lights of very low intensity (15 foot candles), whereas the previous light effects were studied using rather intense natural illumination of greenhouse. A study of the catalase values of these illuminated, genetically green corn seedlings revealed an actual increase in catalase activity upon exposure to light. In other words, there was an increase in catalase activity during the time the chlorophyll was making its appearance. This observation invalidated any thought that, since there is usually a 33% reduction in catalase activity of etiolated genetically green corn seedlings when illuminated by natural light in greenhouse, catalase is somewhat directly converted into chlorophyll. The 33% reduction can only mean that the natural sunlight illumination in greenhouse was too intense for catalase, especially in plants very low in sugar content.

The experiment with low intensity illumination also revealed that there

TABLE V

EFFECT OF LIGHT ON CATALASE ACTIVITY IN CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE		DATE OF MEASUREMENT	AGE OF SEEDLINGS	AMOUNT OF ILLUMINATION	CATALASE ACTIVITY
			days	*hours*	*ml.*
485 (34)	green	1/27/50	13	0	9.04 (2)
	yellow	"	13	0	7.26 (2)
	yellow	"	13	4	7.22 (2)
	green	1/28/50	14	5	10.19 (2)
	yellow	"	14	6	7.52 (2)
485 (45)	green	2/ 2/50	9	A. t. t.*	11.91 (2)
	yellow	"	9	"	5.84 (2)
	green	"	14	"	11.84 (2)
	yellow	"	14	"	3.68 (2)
481 (124)	green	2/ 1/50	18	0	6.42 (2)
	white	"	18	0	5.48 (2)
	white	"	18	7**	5.48 (2)
	white	2/ 2/50	19	24	5.94 (2)
	green	"	19	25	10.14 (2)
	white	"	19	31	5.42 (2)
	white	"	20	48	3.89 (2)
	green	"	20	49	8.02 (2)
481 (150)	green	2/ 7/50	10	A. t. t.*	11.33 (2)
	white	"	10	"	3.82 (2)
	green	2/11/50	14	"	11.84 (2)
	white	"	14	"	4.23 (2)

* All the time.
** Etiolated genetic green seedlings beginning to show presence of chlorophyll.

is photo-decomposition of catalase in yellow and white corn seedlings, but that it was very much slower than under the more intense illumination of sunlight in the greenhouse. The total amount of photo-decomposition of catalase in corn seedlings of comparable ages may not be quite as much under low light intensity.

Etiolated corn seedlings placed in light slowly produce chlorophyll and become green. Invariably the first or oldest leaf is slowest to become green. It may never get green, even though the remainder of the plant does. Measurements of the catalase activity (table VI) showed a significant difference in catalase activity between first leaf and all other leaves. The former was always less in catalase value. The reduction in catalase

TABLE VI

MEASUREMENT OF CATALASE ACTIVITY OF FIRST AND ALL OTHER LEAVES OF ETIOLATED CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	A FIRST LEAVES	B ALL OTHER LEAVES	A/B%
		days	*ml.*	*ml.*	
Commercial	10/19/48	11	6.17 (5)	7.09 (5)	87%
481 (11)	3/11/49	16	8.80 (3)	10.68 (3)	82%

value in the oldest leaf approached the drop in catalase value of etiolated seedlings when they are being illuminated and are developing chlorophyll.

A genotype of corn called zebra which has non-chlorophyll or pale green stripes perpendicular to the leaf axis was analyzed for catalase activity. The portion from the non-chlorophyll or pale green stripes was compared with the alternating green areas as to catalase activity. There really did not seem to be much if any difference (table VII). The light striped por-

TABLE VII

MEASUREMENT OF CATALASE ACTIVITY IN ZEBRA CORN SEEDLINGS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

PEDIGREE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	SOLID GREEN PORTIONS	LIGHT STRIPES
		days	*ml.*	*ml.*
481 (231)	1/21/49	16	7.56 (4)	8.88
481 (17)	2/ 8/49	10	10.23 (3)	8.86 (3) 10.10 (4)
481 (17)	2/11/49	13	8.14 (4)	6.37 (3)

tions must be partially or entirely parasitic on the neighboring green areas. Perhaps the photosynthesized product, sugar, furnished to the pale areas prevents the photo-oxidation of the catalase present there. VON EULER (**2**) reported that white-margined geranium (*Pelargonium zonale*) grown in a nutrient solution supplemented with sugar had a high catalase value in the chlorophyll-deficient part of the leaves, in contrast to a low value for similar tissue from plants growing in nutrient solution not supplemented with sugar. This work of von Euler indicates that photosynthesis might automatically tend to protect the catalase from destruction. Perhaps this was the main reason why catalase value remained high in green plants and why it decreased abruptly when dark-grown albino and yellow seedlings were exposed to light.

A series of hybrid strains of corn from the Pioneer Hybrid Seed Corn Company, arranged from darkest green to lightest green, revealed a pos-

TABLE VIII

MEASUREMENT OF CATALASE ACTIVITY IN PLANTS VARYING IN GREENNESS. CATALASE VALUE IS EXPRESSED AS MILLILITERS OF O_2 IN A FIVE-MINUTE INTERVAL

GREENNESS OF SAMPLE	DATE OF MEASUREMENT	AGE OF SEEDLINGS	CATALASE ACTIVITY
		days	*ml.*
Dark green corn	3/22/49	15	12.89
Glossy dark green corn	3/21/49	14	12.66
Medium dark green corn	3/21/49	14	12.53
Medium green corn	3/21/49	14	12.04
Medium light green corn	3/21/49	14	11.17
Lightest green corn	3/21/49	14	10.96
Dark green coleus	10/20/49	potted plants	12.54
Light green coleus	10/20/49	potted plants	7.29

sible relationship of catalase activity with chlorophyll content (table VIII). The differences in catalase activity among six hybrid strains were small but the trend was consistent. The darkest green strain of corn seedlings had the highest catalase value, and progressively less green strains had progressively less catalase activity. Measurements on quantity of chlorophyll present in each were not made, so the data are insufficient to warrant anything definite between catalase activity and chlorophyll content. Table VIII also gives the catalase values in leaves of intense chlorophyll and dilute chlorophyll genotypes of coleus plants (acquired from Dr. David Rife (1), Ohio State University). Here the difference was quite appreciable, and again the greener one had the higher catalase value.

Summary

1. The catalase activity of leaf tissue of various chloroplast pigment deficient types of corn seedlings growing in a greenhouse was determined by the "catalase tube" method of Knott. The catalase activity of albino seedlings averaged 1.54 ml of O_2 in five-minute interval, that of yellow seedlings 2.83 ml. and that of green seedlings 10.14 ml.

2. These same chloroplast pigment-deficient types of corn seedlings grown in the dark showed a catalase activity which was surprisingly high in all cases and which was approximately the same for all of the three types. The catalase value was usually $\frac{1}{4}$ to $\frac{1}{3}$ lower in green seedlings grown in the greenhouse than in dark-grown albino, yellow and genetic green corn seedlings.

3. When the dark-grown seedlings were exposed to light there was a rapid photo-oxidation of catalase, so that catalase values approached that of similarly aged light (greenhouse) grown albinos, yellow seedlings and green seedlings, respectively.

4. Under artificial light of low intensity (15 fc) there was a small increase in catalase activity when dark-grown genetically green corn seedlings were illuminated, instead of the usual $\frac{1}{4}$ to $\frac{1}{3}$ drop in catalase activity when exposed to brighter light in the greenhouse. Albino and yellow seedlings grown continuously under artificial light of low intensity still showed a much lower catalase value than green corn seedlings of the same age in the same pedigree.

5. Measurements showed a significantly lower catalase activity in the first or oldest leaf of etiolated corn seedlings than in other leaves. This first leaf was also slower in developing chlorophyll when plants were illuminated.

6. In zebra genotype of corn, the light stripes had about the same catalase activity as normal green areas.

7. A series of hybrid strains of corn arranged from darkest green to lightest green showed progressively less catalase activity.

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A STUDY OF POTASSIUM ABSORPTION BY BARLEY ROOTS

LOUIS JACOBSON, ROY OVERSTREET, H. M. KING AND RAYMOND HANDLEY

(WITH SEVEN FIGURES)

Received June 17, 1950

Introduction

As a result of ion absorption studies, both experimental and theoretical, a number of investigators have been led to conclude that the absorption of inorganic ions by cells is accomplished through the agency of chemical forces (**1, 4, 7, 9**). Recently, it has been suggested that ion absorption depends on metabolically produced binding compounds (**3, 4, 7**). Presumably certain of these binding compounds form complexes with cations with the liberation of hydrogen ion, and analogous compounds combine with anions with the liberation of hydroxyl or bicarbonate ions. The foregoing ideas can be expressed in the form of chemical reactions as follows. For cations:

$$HR + M^+ \rightleftarrows MR + H^+,$$

and for anions

$$R'OH + A^- \rightleftarrows R'A + OH^-,$$

where HR and R'OH represent the metabolically produced cationic and anionic binding substances.

The complexes MR and R'A which are formed at the external surfaces of the protoplasm serve as transporting agents, transferring ions from the culture medium to the vacuole. The secretion of the ions into the vacuole must be the result of a chemical alteration of MR and R'A. In order for such a mechanism to be operative, it is necessary that there be a region between the vacuole and the external surface of the protoplasm which is relatively impermeable to free ions, but permeable to the complexes MR and R'A (cf. ROSENBERG, **8**).

The viewpoint that the initial phase of accumulation, *i.e.* the absorption process, is a chemical reaction gives rise to questions which pertain to all chemical reactions; that is, what are the rate determining factors of the reactions, are the reactions reversible, etc. In the present paper, some of these questions have been considered in connection with the absorption reaction for potassium with barley roots.

Experimental material

In conducting extended absorption experiments, a prime prerequisite is reproducibility of the biological material. Low-salt barley plants (**2**) as grown in the greenhouse are admirably suited for absorption studies, but are subject to rather wide fluctuations in fresh weight, root-top ratio and

capacity for absorption of nutrients. These fluctuations are largely the result of seasonal variations in light and temperature conditions (**6**). A simple method for culturing barley roots was therefore adopted which consisted of growing barley seedlings in the dark at a uniform temperature. Roots produced under these conditions compare very favorably with typical low-salt barley roots in their capacity for the absorption of inorganic ions. Moreover, the method has the distinct advantage of being reproducible throughout the year.

The method of culturing barley roots is similar in many respects to that described by ULRICH (**10**). Fifty grams of Sacramento barley seed are treated with 60 ml. of 10% H_2O_2 for 20 minutes. The H_2O_2 solution is drained off without rinsing and the seeds are soaked for 24 hours in 1500 ml. distilled water with continuous aeration. Following this, the seeds are thoroughly washed several times with distilled water and then cultured in a tray of nutrient solution essentially as described by Ulrich. The culture solution used was of the following composition: $Ca(NO_3)_2$, 0.1 m. mol/1; KH_2PO_4, 0.1 m. mol/1; and $MgSO_4$, 0.1 m. mol/1. The temperature is maintained at 26° C and the culture solution replaced three days after setting out the tray. Two days later, the roots are excised and used for experimentation. A yield of approximately 60 g. fresh roots per tray is obtained by this procedure.

Experiments

According to the hypothesis expressed in the introduction, the absorption of potassium by plant roots can be written in terms of the following chemical reaction:

$$HR + K^+ = KR + H^+$$

The reverse of this reaction should lead to the release of potassium ion to external solutions containing hydrogen ion. Some evidence of this was obtained by JENNY and OVERSTREET (**5**) using barley roots and suspensions of hydrogen clays. To investigate this point, aliquots of roots were exposed for three hours to a series of solutions containing varying concentrations of hydrogen ion supplied as HCl. In order to minimize concentration changes in the solution, approximately 20 g. of fresh roots were suspended in 15 l. of HCl solution in each case. The solutions were aerated continuously during the period of treatment. Following the treatment, the roots were washed and analyzed for potassium and other constituents. In order to determine the effect of temperature on the reaction, two identical series were arranged, one at 26° C and the other 0° C. The results for loss of potassium are given in figure 1.

An analysis of the root tissues showed that other plant constituents were also affected by the acid treatment. A summary of the results for calcium, inorganic PO_4, soluble organic PO_4, soluble nitrogen, and dry weight are given in figure 2. The similarity to the potassium outgo curve is evident.

In view of the pronounced outgo of K^+ in solutions of varying H^+ concentration, a determination of the variation of the outgo rate with time was made using 0.005N HCl solutions (pH 2.3). A plot of the percentage loss of K^+ from the roots versus the period of treatment is given in figure 3.

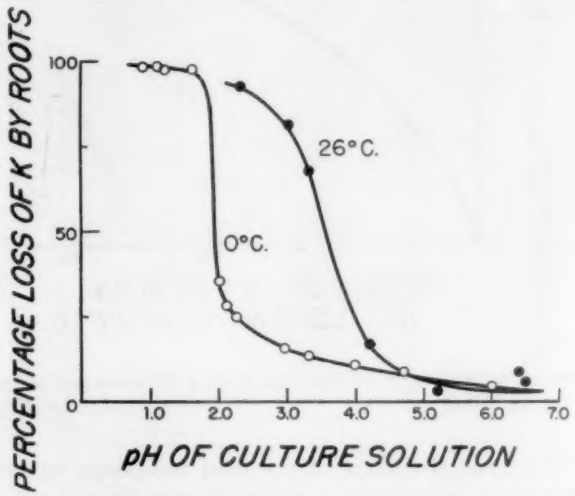

FIG. 1. Loss of potassium by barley roots during a two-hour period of immersion in HCl solutions. For each temperature series (0° C and 26° C) the percentage loss of K is plotted against the pH value of the HCl solution.

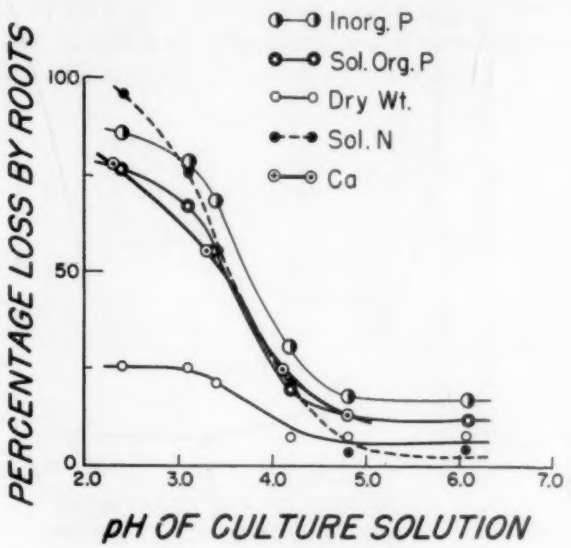

FIG. 2. Loss by barley roots of a number of plant constituents during a two-hour period of immersion in HCl solutions at 26° C. For each constituent the percentage loss is plotted against the pH value of the HCl solution.

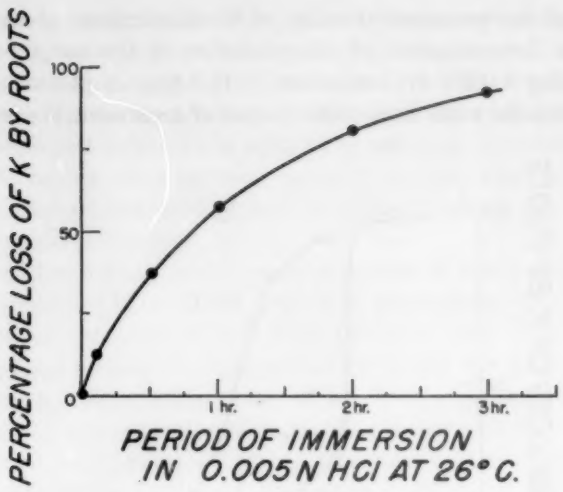

FIG. 3. Loss of potassium by barley roots during different periods of immersion in 0.005N HCl at 26° C.

In order to determine the effect of the acid treatment on the subsequent ability of the roots to absorb K^+, a series of samples were pretreated with 0.005N HCl for varying periods. Following the acid pretreatment, the roots were washed in distilled water and were permitted to absorb K^+ from

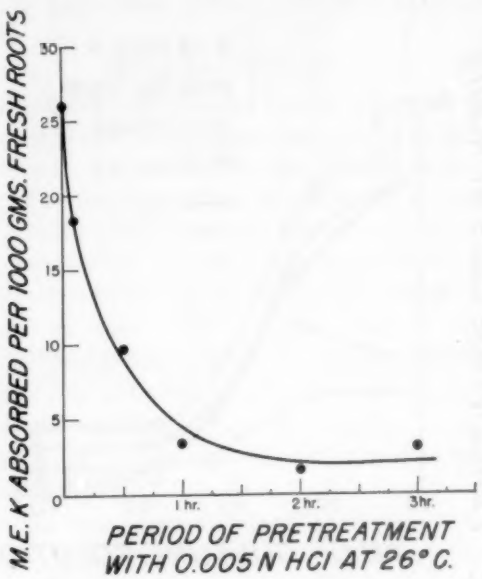

FIG. 4. Decrease in rate of K absorption by barley roots from 0.005N KCl solution at 26° C as a result of the degree of pretreatment of the roots with 0.005N HCl solution at 26° C. Three-hour absorption period.

a 0.005N KCl solution for three hours. The results are summarized in figure 4.

In accordance with the law of mass action the concentration of K^+ in the culture medium should affect the outgo of potassium from the roots, even in the presence of hydrogen ion. To investigate this point, samples of

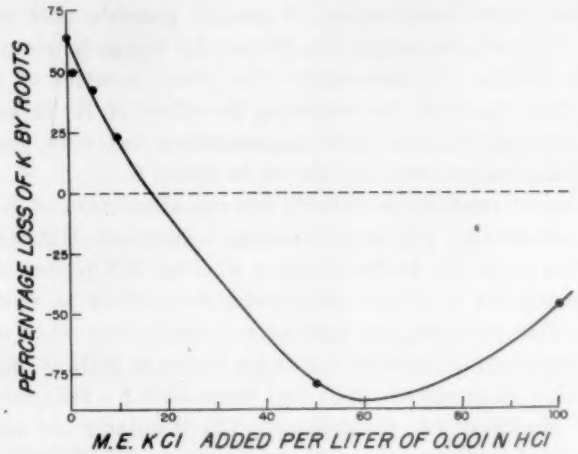

FIG. 5. Effect of added KCl on the loss of potassium by barley roots in 0.001N HCl solutions at 26° C. Absorption period: two hours.

barley roots were exposed for two-hour periods to a series of HCl solutions, containing 0.001N HCl and varying amounts of KCl. The data are presented in figure 5, in which the percentage loss of potassium from the roots is plotted against the KCl concentration of the solution. The experiment

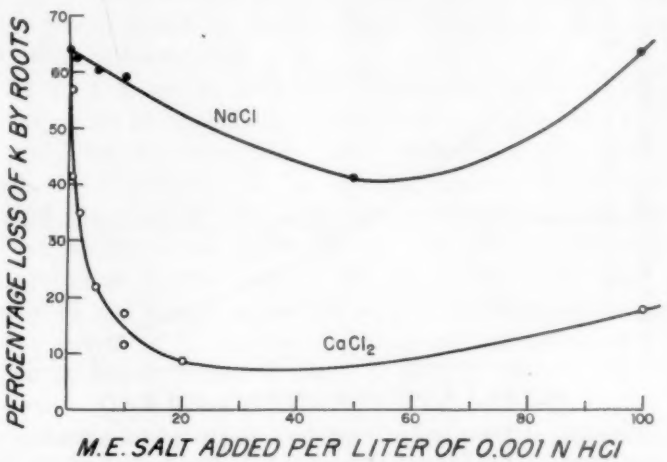

FIG. 6. Effect of added NaCl and $CaCl_2$ on the loss of potassium by barley roots in 0.001N HCl solution at 26° C. Two-hour absorption periods.

was conducted in the presence of 0.001N HCl because in the absence of KCl, approximately 63% of the potassium is lost in a two-hour immersion period. In figure 5, negative values of the percentage potassium lost correspond to a net accumulation of the element.

Because of the possibility that the absorption reaction for potassium may not be an isolated reaction, and may be linked indirectly to reactions involving other plant constituents, it seemed possible that the outgo of potassium in HCl solution might be affected by the presence of other ions in the culture media. To investigate this point, a series of experiments analogous to that described for studying the effect of K^+ in acid solution, were carried out at the same acid concentration but with Ca^{++} and Na^+. The results of this experiment are shown in figure 6.

In the chemical reaction postulated for the absorption of K^+, the existence of a metabolically produced binding compound HR was assumed. This assumption gives rise to the question whether HR represents a specific binding compound for K or can also bind other cations as well. In order to investigate this problem, the following experiments were carried out: (1) Barley roots were immersed for three hours in 0.005N KCl solutions to which varying amounts of NaCl had been added. Following this, the amount of K^+ absorbed was determined. (2) Similarly the absorption of K^+ was determined in the presence of varying amounts of $CaCl_2$. The data for the two experiments are summarized in figure 7. A third experiment was designed to determine the replaceability of K in the complex KR. In

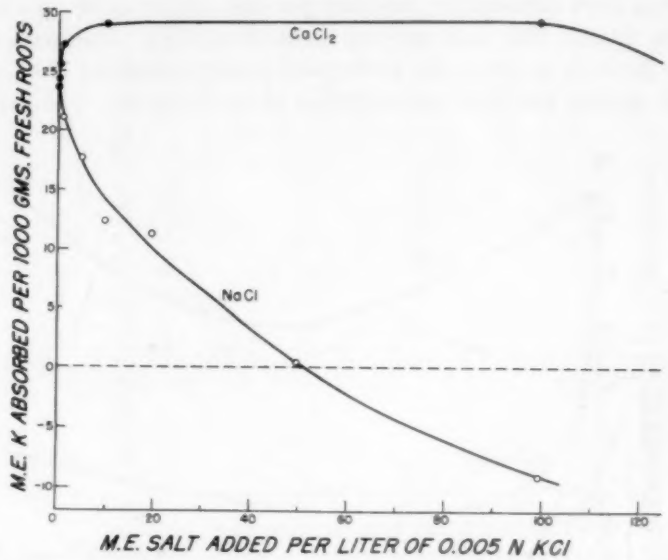

FIG. 7. Effect of additions of NaCl and $CaCl_2$ on the rate of potassium absorption by barley roots from 0.005N KCl solutions at 26° C. The portion of the $CaCl_2$ curve for additions of $CaCl_2$ above 100 M.E. per liter has been omitted. Three-hour absorption periods.

TABLE I

LOSS OF POTASSIUM BY ROOTS AS A RESULT OF IMMERSION IN VARIOUS SALT SOLUTIONS FOR TWO HOURS AT 26° C

CULTURE SOLUTION	PER CENT. K LOST BY ROOTS
0.01N $CaCl_2$	3.9
0.01N $MgCl_2$	2.4
0.01N NaCl	2.4
0.01N NH_4Cl	17.9
0.01N HCl	95.0

this experiment, barley roots were exposed for three hours to 0.01N solutions of various cations. After a three-hour period of immersion, the roots were analyzed for their K content. The observed percentage loss of K in each of the solutions is given in table I.

Discussion

The data obtained support the idea that the uptake and loss of potassium in excised barley roots may be described by the following reversible reaction:

$$HR + K^+ \rightleftarrows KR + H^+$$

According to the reaction as written, the direction of the process should be determined by the amounts of H^+ and K^+ in the culture solution. This is borne out by the data of figures 1 and 5. That the process is reversible is seen in the data of figure 5. Below a molar ratio of K^+ to H^+ of 17:1, potassium was lost from the roots to the culture solution, but, when potassium in the culture solution exceeded this ratio, it was accumulated by the roots. In considering this particular system involving equilibrium between K^+ and H^+ the results strongly indicate that in the estimation of the ability of a plant to absorb potassium from a culture medium, the activities of both K^+ and H^+ must be considered.

From figure 1, it can be seen that in pure HCl solutions at 26° C a critical point occurs in the neighborhood of pH 4.5, below which potassium is very rapidly lost from the roots. At 0° C, however, the similar critical pH is much lower, namely, pH 2.5. On the basis of the hypothesis presented, loss of potassium by the plant to the acid solution must be accompanied by an absorption of hydrogen ions. Moreover, absorption of H^+ should be governed by the same metabolic influences as other cations. Since absorption is a metabolic process and hence greatly affected by temperature, the uptake of H^+ (or loss of potassium) should occur to a much larger extent at 26° C than at 0° C. The data of figure 1 are consistent with this view.

However, it should be pointed out that whenever conditions in the culture medium strongly favor the absorption of hydrogen ion, pronounced and undoubtedly injurious effects are produced in the tissue. The curves

of figures 2 and 4 show that these effects are manifested by a general exodus of cellular constituents and a reduced capacity of the roots for subsequent absorption.

In connection with figure 5, it has already been pointed out that the loss of potassium and the absorption of hydrogen in acid solution can be prevented by the addition of KCl. An analysis of the same samples for calcium indicated that not only is the presence of KCl effective in decreasing the loss of potassium in acid solutions but also in decreasing the loss of calcium. For example, the calcium loss in pure 0.001N HCl was 58.2%; when 50 m. eq/l. of KCl was added to the HCl solution, the loss of calcium decreased to 25.3%. Results to be published later show that the losses of other plant constituents are similarly affected by the addition of KCl.

Inter-relationships between hydrogen, calcium and potassium are further borne out by figure 6. The addition of $CaCl_2$ to acid solutions greatly decreased the loss of potassium. A minor decrease in the loss of potassium occurred as a result of adding NaCl to the 0.001N HCl solution. This decrease was very likely due to a simple competition between Na and H ions in an absorption reaction, since sodium is rapidly accumulated by these roots. However, the effect of calcium appears too great to be explained on the basis of a simple competitive relationship, since calcium is absorbed rather slowly by the roots.

Although it is presumed that the binding compound HR is produced by the metabolic oxidation of carbohydrate, it should be stated here that the substance HR is not necessarily an organic acid but rather a compound which forms an undissociated complex with cations and at the same time releases H^+. It has not yet been established whether the term HR represents a specific compound for each cation or a single compound which serves for all cations. If the former were true, it might be expected that the presence of other cations in the culture medium would not greatly influence the absorption of a given cation. If the latter were true, then a marked competition should exist between the cations for the HR produced. Figure 7 indicates that the absorption of potassium is markedly decreased in the presence of increasing amounts of NaCl, suggesting that the same binding compound serves for the absorption of both sodium and potassium. Also, it appears from the data of table I that the potassium of the already formed complex KR may be replaced by other cations, particularly H^+ and NH_4^+.

The addition of $CaCl_2$ significantly increases the absorption of potassium. This observation confirms the findings of VIETS (**11**). It would appear that either calcium and potassium do not compete for the same binding compound or more likely that calcium occupies a special role, possibly being a co-factor in the formation of HR.

Summary

The absorption of potassium by barley roots has been studied in the

presence of H^+ and other cations. An attempt has been made to explain the data on the basis of the following reaction.

$$HR + K^+ \rightleftarrows KR + H^+$$

where HR represents a metabolically produced binding compound.

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THE ACTION OF INHIBITORS ON WATER UPTAKE BY POTATO TISSUE

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(WITH ONE FIGURE)

Received June 7, 1950

Introduction

The potato tuber and other storage tissues have been used for more than 30 years in the study of permeability and salt accumulation. However, until recently, less attention was paid to the uptake of water by these tissues, though both Stiles and Steward noted that when thin slices are used the water uptake is considerable. In 1938 REINDERS (**5**) showed that the prolonged uptake of water is a strictly aerobic process, and concluded (**6**) that the energy of respiration "creates the condition" causing water uptake. She also found that the uptake is promoted by low concentrations of auxin. This observation, which has been confirmed by several workers, suggested that a more careful study might shed some light on the mechanism of auxin action. The work reported here, in summary form, deals only with the relation between water uptake and metabolism as shown by means of several metabolic inhibitors.

Methods

A cylinder of tissue is cut from a potato (var. Katahdin) previously stored at about 14° C. Discs 1 mm. thick and 1 cm. in diameter are sliced from this with a hand microtome. The discs are washed for 15 minutes in running tap-water, allowed to stand in shallow water for 24 hr., blotted and weighed in groups of ten discs, and then transferred to the experimental apparatus. In the past the usual procedure has been to place the discs in a relatively large volume of water, through which air was bubbled (**7, 6**). However, in studies of the growth of sections of Avena coleoptiles (**10**) it was found that when the sections were mounted so as just to break the surface of the liquid they grew as rapidly as if they were submerged and vigorously aerated. Accordingly, we have mounted the potato discs so as to break the surface of a shallow unaerated solution. The apparatus consists of a fine net stretched over a large glass ring, 8 cm. in diameter, resting in a Petri dish. After weighing, the discs are placed on the net, the solution added up to the appropriate level (40 to 50 cc.); and the dishes transferred to a dark room at 25° C. Discs are usually reweighed at two-day intervals.

The principal justification for this simplified method is the fact that the phenomena described in the literature with the more complex method can be duplicated. The absolute values of the water uptake obtained by

this method are within the range of values (which vary widely) reported by other investigators.

Results

Results of a typical experiment are given in table I. A duplicate experiment gave almost identical results. The uptake of water, as measured by the increase in fresh weight, continues for six days in those discs which are breaking surface. In comparable experiments the percentage increase

TABLE I

THE UPTAKE OF WATER BY POTATO TISSUE AS MEASURED BY INCREASE IN FRESH WEIGHT. BLOTTED FRESH WEIGHTS IN GRAMS OF TEN DISCS (1 CM. × 1 MM.) IN EACH SERIES

DAYS FROM START*	0	2		4		6	
Solution	WEIGHT	WEIGHT	PER CENT. INCREASE	WEIGHT	PER CENT. INCREASE	WEIGHT	PER CENT. INCREASE
Water, breaking surface	1.23	1.30	*5.7*	1.335	*8.5*	1.345	*9.4*
Water, submerged	1.14	1.16	*1.8*	1.17	*2.6*	1.18	*3.5*
IAA (10 mgm./l.)	1.20	1.275	*6.3*	1.345	*12.1*	1.38	*15.0*
NaN_3 (10^{-4} M, pH 6.0)	1.14	1.15	*0.9*	1.155	*1.3*	1.17	*2.6*

* The experiment starts when the discs are put into the test solution, *i.e.* after 24 hours' washing.

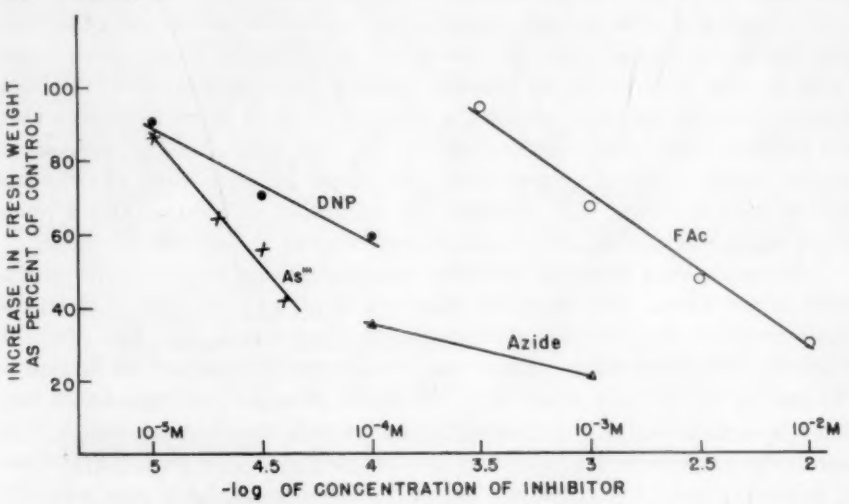

FIG. 1. The increase in fresh weight, as per cent. of that of controls in distilled water, of 1 cm. × 1 mm. potato discs after four days in solutions of enzyme inhibitors. Each point represents the weight of 10 discs and in addition is the mean of the following number of repetitions: for three to five dinitrophenol (DNP), four; for arsenite, four; for azide, four to six; for fluoroacetate, four. (January–April 1950). pH of solutions 6.0–6.2, except DNP 5.5.

after six days has varied from 7 to 13%. Discs submerged in the same volume of water, under a depth of about 5 mm., show less than half the water absorption. Indole-acetic acid in a concentration of 10 mgm./l. increases the water uptake considerably. These facts are in accord with Reinders' findings on the effect of anaerobic conditions and auxin. On the other hand, a 10^{-4} M solution of sodium azide very strongly reduces the water absorption throughout the six-day period. The discs remain in excellent condition, although slightly paler than the controls. In none of the cases reported here did any of the discs show signs of injury or bacterial decomposition.

The results of a large number of similar experiments with four different inhibitors are combined in summary form in figure 1. The increase in fresh weight, as per cent. of that of the control, is plotted against the negative logarithm of the concentration of inhibitor (in distilled water). The expression of fresh weight as per cent. of that of the control allows the comparison of experiments in which the absolute water uptake of the sections varies. Although the range of effective concentrations differs considerably, in each case there appears to be a roughly linear relationship between water uptake and the log of the inhibitor concentration. Clearly, the aerobic uptake of water by potato discs can be prevented by a variety of enzyme inhibitors.

Discussion

The inhibitors used have been shown to act on a number of different processes: azide acts on cytochrome oxidase, arsenite inhibits enzymes containing essential –SH groups, fluoroacetate acts as an antagonist of acetate and blocks its metabolism via the Krebs cycle, while dinitrophenol apparently acts by uncoupling phosphorylation from respiration. The fact that all these compounds inhibit the increase in fresh weight suggests that the general respiratory metabolism of the cell underlies the process of aerobic water-uptake.* Specifically, the data indicate that cytochrome oxidase, one or more –SH enzymes, the oxidation of acetate, and a phosphorylating mechanism are linked in some way to the uptake of water.

The mechanism whereby oxidative metabolism can control water movement is not clear. The work of Brauner *et al.* (**1**) suggests that an osmotic gradient may be the ultimate controlling factor, and the effect of auxin in promoting water uptake was interpreted in this way by Reinders (**6**) and by Commoner *et al.* (**2**). However, it seems not improbable that there is a metabolically controlled system which accumulates water in a manner analogous to the way ions are accumulated. Indeed, suggestions of this sort, under the name of "active" water uptake, have been made by several authors (**8, 4**; cf. also (**3**)).

* In Levitt's researches (Plant Physiol. 23: 505–515. 1948) on auxin-induced water uptake it may be seen that cyanide does not inhibit even the uptake in the absence of auxin. Since this is in clear conflict with the above data obtained with four different inhibitors, it is evident that the case of cyanide is a special one which calls for more detailed consideration.

All of the compounds used have been shown to act as inhibitors of the growth of sections of Avena coleoptiles and Pisum stems, in presence of auxin (**9**). Iodoacetate, which has been extensively used in that connection, was less satisfactory in the present experiments because of apparent injury accompanying moderate inhibition. A comparison of the concentrations of the various inhibitors which produce a 50% inhibition of both growth and water uptake shows a striking similarity, as shown in table II.

TABLE II

CONCENTRATIONS OF INHIBITORS CAUSING 50 PER CENT. INHIBITION OF GROWTH AND WATER UPTAKE

	CONCENTRATION X 10^{-5} M OF:—			
	ARSENITE	DINITROPHENOL	FLUOROACETATE	IODOACETATE
Growth of coleoptiles	1	4	300	4
Growth of peas	10	20	600	60
Water uptake by potatoes	3	25	300	(30)*

* The figure is approximate owing to varying degrees of injury.

It may be suggested that those metabolic processes which are intimately linked with growth are the same as those linked with aerobic water uptake. Both, indeed, are phenomena of cell enlargement.

Summary

A simplified method for the measurement of the aerobic water uptake by discs of storage tissue is described.

The water uptake can be inhibited quantitatively by azide, dinitrophenol, arsenite and fluoroacetate, all of which are inhibitors of oxidative enzyme systems and also of growth.

Implications of the results with respect to the link between respiration and water absorption are briefly discussed.

The authors wish to thank Dr. W. C. Libby of the University of Maine Agricultural Experiment Station for a regular supply of potatoes, var. Katahdin.

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THE PHOSPHORUS UPTAKE OF *ANDROPOGON ISCHAEMUM* L. AT VARIOUS STAGES OF DEVELOPMENT

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(WITH FIVE FIGURES)

Received May 15, 1950

Introduction

The general subject of plant nutrition, and specific considerations of nutrition as related to development have been comprehensively reviewed by LOEHWING (**14, 15**) and HOAGLAND (**10, 11**). An examination of the literature will reveal, however, that relatively little attention has been given to the relationships between specific nutritional factors and development in grasses other than those falling within the category of crop plants. The investigation of certain of these relationships is now desirable because of increasing attention to range grass problems. In this initial investigation phosphorus nutrition was selected because in the range land regions phosphorus deficiencies are common. Studies of phosphorus nutrition have been numerous but only a few have been related to developmental factors. The relation of phosphorus absorption and nitrogen supply has been studied by BREON, *et al.* (**5**) in the tomato and by WILLIAMS (**19**) in oats. Williams noted a decrease in phosphorus in the stem and leaf and an increase in phosphorus in the inflorescence at the time of flowering. With reference to the onset of flowering BAKHUYZEN'S (**2**) conclusion that flowering marks the transition in plants from an anabolic to a catabolic state should be noted. Another observation of Bakhuyzen that phosphorus is excreted by plants under certain conditions was also noted earlier by HORNBERGER (**13**), WILFARTH, *et al.*, (**18**), BURD (**7**), PENSTON (**17**) and ACHROMEIKO (**1**). This loss of phosphorus at certain stages was revealed in several experiments preliminary to the one reported here and is one of the subjects of this investigation.

This investigation had as its purpose the evaluation of phosphorus absorption by King Ranch bluestem, a strain of *Andropogon ischaemum* L., at various stages of its growth and development. A knowledge of at what period or periods in the growth cycle phosphorus is absorbed most rapidly is of obvious importance in attempts to discover the specific functions of this element in growth and development. This knowledge also has an important bearing upon practical considerations such as the proper time for the application of phosphate-containing fertilizer. A study of certain aspects of the relationship between soil phosphate and the phosphorus nutrition of this grass species has been made by NOGGLE and WYND (personal communication).

Materials and methods

Dehusked seeds of *Andropogon ischaemum* L. were sown in November, 1948, in acid-washed quartz sand in one-gallon crocks. This planting constituted the first of a series. The seeds were sown about one inch deep and the crocks were covered with glass plates to prevent drying. At the time of sowing 500 ml. of Wolkoff's modification of Shive's solution (**21**) containing approximately 40 mg. of phosphorus in the form of KH_2PO_4 were added to each crock. Trace element solutions A and B of HOAGLAND and ARNON (**12**) were used with the nutrient solution. The plants were watered daily with tap water and weekly with nutrient solution. Samples consisting of whole plants were taken initially at 2–3 day intervals and later at weekly intervals. Until the seedlings were 14 days of age, each sample consisted of 200 seedlings chosen at random. As the seedlings grew larger the number of plants in a sample was reduced progressively to a minimum of 20. The seedlings were removed, washed free of sand and nutrient solution, dried in a forced air drying oven at 85°–90° C, and weighed. In the case of the smaller seedlings, the whole sample, weighing not more than 0.1 gm., was used in the chemical analyses. For larger plants, the dried sample was ground in a Wiley mill to pass through a 60 mesh sieve, and a 0.1 gm. portion of the uniform material was taken for analysis. Most samples were divided into roots and shoots and the dry weights and phosphorus content of these two fractions were determined separately. A few plants were further divided into young and old parts of the shoots and roots.

Each sample was wet-ashed on a hot plate with concentrated sulphuric acid and 30% hydrogen peroxide, following WOLF'S (**20**) procedure. The solution was then made up to 50 ml. with Morgan's universal extracting solution (**16**). Appropriate aliquots (5.0–8.0 ml.) of the plant extract were taken for total phosphorus determination by the Fiske-Subbarow method (**9**), using a Cenco-Sheard photelometer and a blue filter. Photometric readings were converted into micrograms of phosphorus with the aid of a standard curve prepared with known amounts of phosphorus.

Analysis of a second series of plants was started in January, 1949. These plants were thus exposed to different day-lengths than the first set. They were watered with the nutrient solution twice a week to determine whether a more abundant supply of phosphorus would increase phosphorus absorption. In the second set, weight and phosphorus determinations were made on larger samples (up to 1000 plants) and statistical analyses were made to see whether sampling errors had been involved in the results obtained with the first set. Otherwise plants of the second series were handled in the same manner as those of the first series. Another series of plants parallel to series 1 was sampled and dissected at intervals for morphological and anatomical studies. While the results of these studies are being reported in a separate paper, certain pertinent data have been included here.

Results

The dry weights of the seedlings continued to decrease slightly for the first seven days (table I). After this small initial loss in weight, the seedlings gained in dry matter rapidly. In series 1 a more rapid dry weight increase was observed during the 37–42 day period when the internodes started to elongate. There was a definite decrease in the dry weight increment at the time of flowering. The shoot/root ratio decreased grad-

TABLE I

DRY WEIGHTS AND SHOOT/ROOT RATIOS OF *Andropogon ischaemum* AT VARIOUS DEVELOPMENTAL STAGES. FOR THE STAGE OF MORPHOLOGICAL DEVELOPMENT AT VARIOUS SAMPLING AGES SEE FIGURE 5

AGE IN DAYS	NUMBER OF PLANTS SAMPLED	DRY WEIGHT (MG. × 10^{-1})			SHOOT/ROOT RATIO	MORPHOLOGICAL STAGE
		ROOT	SHOOT	TOTAL		
SERIES 1						
0	200	.....	.....	3.2	.....	
2	"	.....	.....	3.1	.....	
3	"	.....	.....	2.5	.....	
5	"	.....	.....	2.5	.....	
7	"	.....	.....	2.5	.....	
9	"	.....	.....	2.8	.....	
13	"	.....	.....	5.2	.....	
19	100	2.0	10.4	12.4	5.2	
22	"	4.2	19.4	23.6	4.6	
25	"	9.1	39.2	48.3	4.3	
28	"	12.2	53.7	65.9	4.4	
32	"	19.7	72.2	91.9	3.6	
37	"	30.2	97.4	127.6	3.2	elongation of internodes
42	50	83.9	335.6	419.5	4.0	
46	25	224.9	663.4	888.3	3.0	tillering
58	"	334.6	1311.4	1646.0	3.9	flowering
63	"	452.1	1432.8	1884.9	3.2	
70	"	649.4	1975.3	2624.7	3.2	
77	"	823.8	2155.0	2978.8	2.6	
85	"	1255.0	4145.5	5400.0	3.3	
100	"	1734.0	5056.0	6790.0	2.8	
SERIES 2						
0	5 × 200	.....	.....	3.0 ± 0.06*	.....	
2	4 × 200	.....	.....	2.9 ± 0.25	.....	
4	3 × 100	.....	.....	2.7 ± 9.03	.....	
7	4 × 100	.....	.....	2.7 ± 0.17	.....	
13	4 × 100	.....	.....	5.8 ± 0.08	.....	
21	2 × 100	5.9	21.5	27.4	3.7	
28	2 × 100	9.5	35.2	44.7	3.7	
35	100	37.9	103.5	141.4	2.7	
42	2 × 50	91.4	261.4	352.8	2.8	
49	50	273.1	700.5	973.6	2.6	
52	25	355.0	1083.6	1438.6	3.0	
56	20	370.8	1305.6	1676.4	3.5	
63	40	460.2	1807.2	2267.4	3.9	flowering
70	20	714.0	2354.0	3068.0	3.4	
77	40	636.0	3175.0	3811.0	.....	
84	20	775.0	4449.0	5124.0	.....	

* Standard error.

ually as development proceeded, indicating that root growth became more rapid than shoot growth in the later stages (table I). This change is more uniform and more pronounced in plants of the first series than those in the second series.

The growth increments shown in figure 1 were calculated by dividing

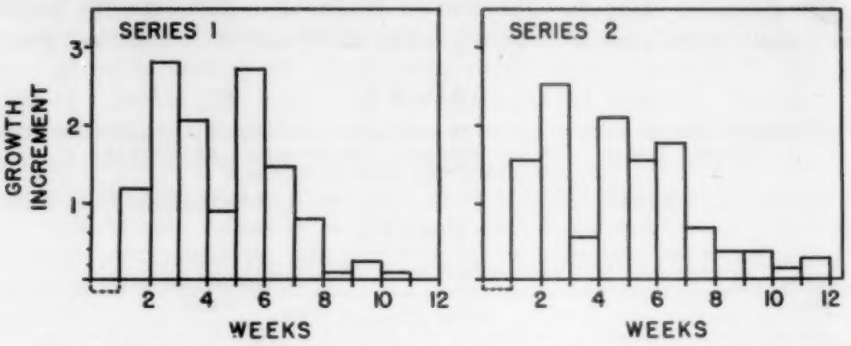

FIG. 1. Weekly growth increments of *Andropogon ischaemum*. For stage of morphological development refer to figure 5. Series 1 plants were grown in the greenhouse from November, 1948 through January, 1949; series 2 plants from January through March, 1949.

the weekly increase by the weight at the beginning of the week. They thus represent the amount of dry matter formed per unit of material present at the beginning of the period. In both series 1 and 2 there appear to be two distinct growth maxima. The first maximum occurs in the second to third week in both series. The second maximum occurs in the fifth to sixth week in series 1 and in the fourth to seventh week in series 2. In both series the first maximum is slightly greater than the second.

The seedlings took up very little phosphorus in the first seven days (table II), during which period little increase in dry weight occurred. After the ninth day there was a progressive, but not steady, increase in the uptake of phosphorus except for one period between the 46th and 63rd days in series 1 and 52nd and 56th days in series 2 when there was an actual decrease in the amount of phosphorus present in the plants. This decrease in the amount of phosphorus was not accompanied by any decrease in the dry weight of the root (see table I). It is, therefore, not the result of a mere loss of root material during the process of sampling, but rather there is an actual loss of phosphorus.

Relatively more phosphorus was present in the root than in the shoot at the later stages of development than at the earlier stages. This is revealed by the decrease of the shoot phosphorus/root phosphorus ratio (last column of table II). This change is partly caused by the more rapid growth of the root and is partly a result of the fact that the concentration of phosphorus in the root did not undergo as great a drop as that in the shoot at the later stages of development.

The figures on phosphorus-absorbing efficiency (fig. 2) were obtained

by dividing the weekly increment of phosphorus by the dry weight of the whole plant at the beginning of the period.

TABLE II

PHOSPHORUS UPTAKE IN *Andropogon ischaemum* IN RELATION TO AGE. CALCULATED ON THE BASIS OF DRY WEIGHT OF ROOT, SHOOT AND TOTAL PLANT

AGE IN DAYS	PHOSPHORUS PER PLANT (P.P.M.)			SHOOT PHOSPHORUS/ ROOT PHOSPHORUS RATIO
	ROOT	SHOOT	TOTAL PLANT	
Series 1				
0	.....	.....	2.2	.....
2	.....	.....	2.5	.....
3	.....	.....	2.5	.....
5	.....	.....	2.8	.....
7	.....	.....	2.7	.....
9	.....	.....	2.8	.....
13	.....	.....	3.4	.....
19	.....	.....	6.7	.....
22	1.3	12.2	13.5	9.4
25	3.1	31.7	34.8	10.2
28	5.0	38.1	43.1	7.7
32	5.9	45.5	51.4	7.7
37	13.0	93.1	106.1	7.7
42	36.1	299.0	335.1	8.2
46	105.7	636.5	742.2	6.0
58	103.7	472.0	575.7	4.5
63	90.4	458.5	548.9	5.0
70	136.3	671.6	807.9	4.9
77	181.6	858.4	1040.0	4.7
85	226.5	1308.0	1534.5	5.7
100	309.2	1570.0	1879.2	5.0
Series 2				
0*	.....	.....	1.5	.....
1	.....	.....	1.5	.....
2	.....	.....	2.1	.....
4	.....	.....	1.9	.....
7	.....	.....	2.0	.....
14	.....	.....	3.8	.....
21	1.5	9.0	10.5	6.0
28	3.1	19.0	22.1	6.1
35	12.5	46.6	59.1	3.8
42	44.1	175.1	219.2	4.0
49	92.8	313.2	408.0	3.4
54	81.6	682.7	764.3	8.4
56	118.4	561.1	679.5	4.7
63	151.8	777.0	928.8	5.1
70	235.6	1059.3	1294.9	4.5
77	148.2**	1240.2	1388.4	8.4
84	201.5	1512.7	1714.2	.....

* In the case of ungerminated seeds (0 days) in series 2, determination of phosphorus was made on soaked and subsequently dried seeds. In series 1 the seeds used were not soaked. The lower value obtained in series 2 is therefore believed to be due to the loss of material during the process of soaking. This difference is also shown in the dry weight data presented in table I.

** See root weight in table I.

Like the growth increment graphs, the phosphorus-absorbing efficiency graphs also exhibited two maxima, occurring in the second to third week and fifth to sixth week in both series.

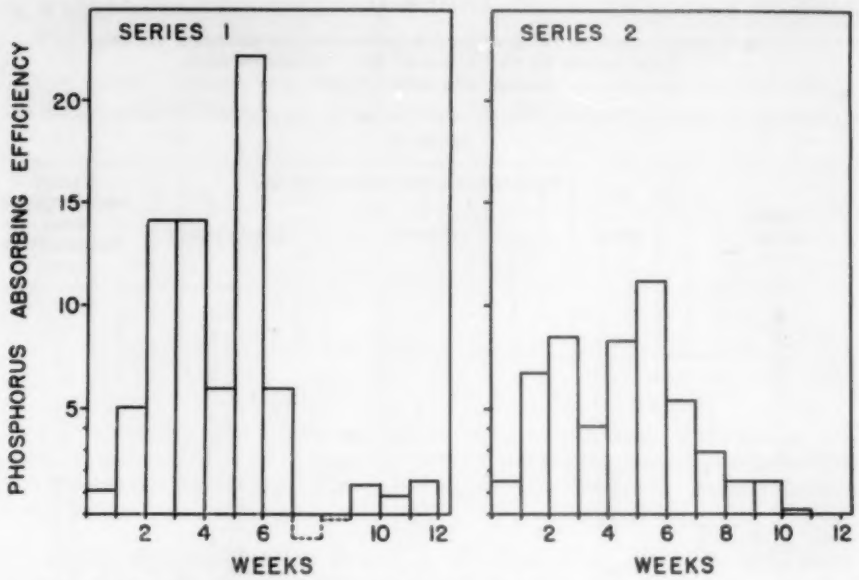

FIG. 2. The weekly phosphorus-absorbing efficiency of *Andropogon ischaemum* based on total plant weight. For stage of morphological development refer to figure 5. Series 1 plants were grown in the greenhouse from November, 1948 through January, 1949; series 2 plants from January through March, 1949. Phosphorus-absorbing efficiency was calculated by dividing the weekly increment of phosphorus by the dry weight of the whole plant at the beginning of the period.

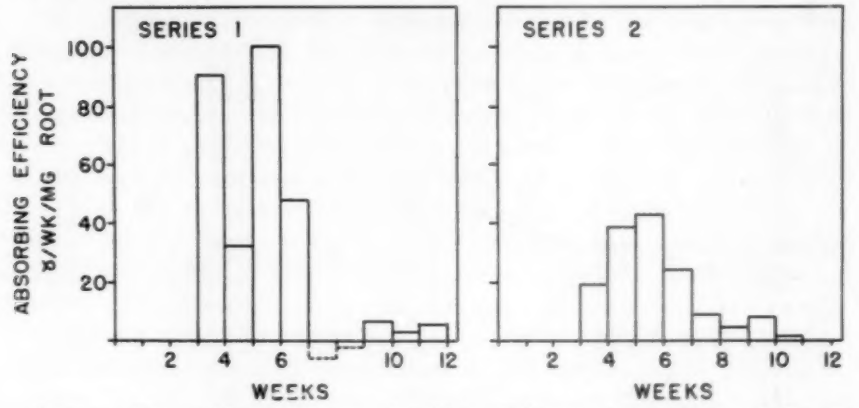

FIG. 3. The weekly phosphorus absorbing efficiency of *Andropogon ischaemum* based on root weight. For stage of morphological development refer to figure 5. Series 1 plants were grown in the greenhouse from November, 1948 through January, 1949; series 2 plants from January through March, 1949. Phosphorus-absorbing efficiency was calculated by dividing the weekly increment of phosphorus for the whole plant by the root weight at the beginning of the period.

Since the root is primarily concerned in the process of absorption, it was thought desirable to express the absorbing efficiency values in terms of the root weight alone (fig. 3). The values for the first three weeks are lacking because at that stage too many seedlings would have been needed to get reliable root weights. These graphs bear a close resemblance to those of figure 2, in having a maximum in the fifth to sixth week and in the reduction of magnitude after the seventh week (negative values were

TABLE III

THE CHANGE OF PHOSPHORUS CONCENTRATION IN *Andropogon ischaemum* PLANT PARTS WITH AGE. R = ROOT; S = SHOOT; R_u = UPPER OR BASAL FIVE-INCH PORTION OF ROOT; R_l = LOWER OR APICAL PORTION OF ROOT; S_o = OLD FLOWERING SHOOT; S_y = YOUNG VEGETATIVE TILLER. MGS. P/MG. DRY WEIGHT

AGE IN DAYS	WHOLE PLANT	R	S	R_u	R_l	S_o	S_y
			SERIES 1				
0	6.9	.....	.....	.....	.....	.....	.....
2	8.1	.....	.....	.....	.....	.....	.....
3	9.9	.....	.....	.....	.....	.....	.....
5	11.1	.....	.....	.....	.....	.....	.....
7	10.7	.....	.....	.....	.....	.....	.....
13	6.6	.....	.....	.....	.....	.....	.....
16	5.7	.....	.....	.....	.....	.....	.....
19	5.4	.....	.....	.....	.....	.....	.....
22	5.7	3.1	6.3	.....	.....	.....	.....
25	7.2	3.4	8.1	.....	.....	.....	.....
28	6.5	4.1	7.1	.....	.....	.....	.....
32	5.6	3.0	6.3	.....	.....	.....	.....
37	8.3	4.3	9.6	.....	.....	.....	.....
42	8.0	4.3	8.9	.....	.....	.....	.....
46	8.4	4.7	9.6	.....	.....	.....	.....
58	3.4	3.1	3.6	.....	.....	.....	.....
63	2.9	2.0	3.2	.....	.....	.....	.....
70	3.1	2.1	3.4	.....	.....	.....	.....
77	2.9	2.2	3.2	1.6	2.7	3.0	3.8
85	2.9	1.8	3.2	1.0	2.3	2.9	3.5
92	2.5	1.8	2.6	1.6	2.2	2.6	2.9
100	2.7	1.8	3.1	1.4	2.2	2.9	3.4
			SERIES 2				
0	4.8	.....	.....	.....	.....	.....	.....
1	4.5	.....	.....	.....	.....	.....	.....
2	7.4	.....	.....	.....	.....	.....	.....
4	8.1	.....	.....	.....	.....	.....	.....
7	7.2	.....	.....	.....	.....	.....	.....
14	6.6	.....	.....	.....	.....	.....	.....
21	3.9	2.6	4.2	.....	.....	.....	.....
28	4.9	3.3	5.4	.....	.....	.....	.....
35	4.2	3.3	4.5	.....	.....	.....	.....
42	6.2	4.5	6.7	.....	.....	.....	.....
49	4.2	3.4	4.5	.....	.....	.....	.....
52	5.3	2.3	6.3	.....	.....	.....	.....
56	4.0	3.2	4.3	.....	.....	.....	.....
63	4.1	3.3	4.3	.....	.....	.....	.....
70	4.2	3.3	4.5	.....	.....	.....	.....
77	3.6	2.3	3.9	.....	.....	.....	.....
84	3.2	2.6	3.4	.....	.....	.....	.....

obtained from the seventh to ninth week in series 1). The decrease in the absorbing efficiency is presumably related to such developmental factors as metabolic changes associated with maturation.

The variation of total phosphorus content on a dry weight basis in the different plant parts is shown in table III. In both series, the highest figures were obtained during the early stage of germination when the young seedlings were taking up phosphorus and losing dry weight simultaneously. After reaching this peak the phosphorus content decreased gradually except when at about the sixth week a second high point was reached. The phosphorus content in the shoot is about one and a half to two times as high as in the root on the unit dry weight basis. The apical portion of the root contained more phosphorus than the basal portion (the basal five-inch portion). Young vegetative tillers were invariably higher in phosphorus than old flowering shoots. Plants in series 2 maintained a lower phosphorus content in the pre-floral period than those in series 1 despite the more frequent addition of nutrient solution to the medium. This is probably an expression of the "dilution" phenomenon noted by WILLIAMS (**19**). Just prior to flowering the phosphorus content of the plants of series 2 increased rapidly with the result that the phosphorus content of these plants exceeded that of the series 1 plants at the end of the experiment.

To show the relationship between phosphorus uptake and dry weight in-

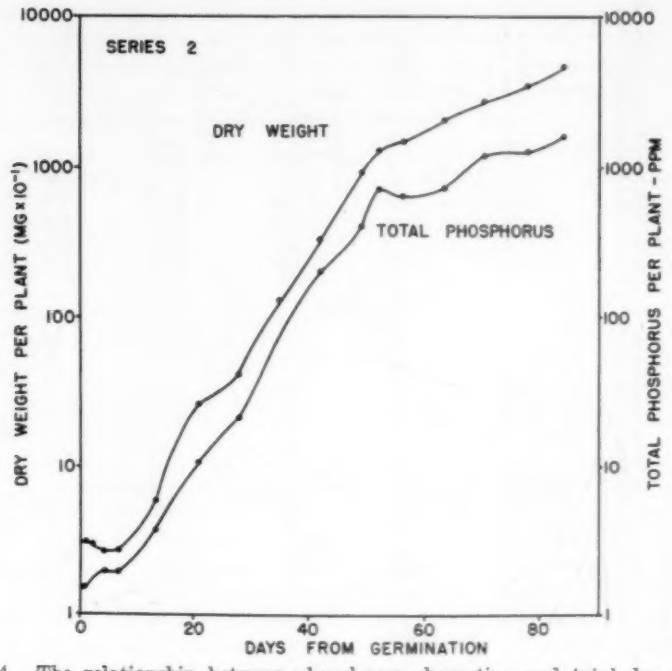

FIG. 4. The relationship between phosphorus absorption and total dry weight of *Andropogon ischaemum*.

crease for series 2 both dry weights and total phosphorus, on a per-plant basis, were plotted against the days from germination (the ordinates being on a logarithmic scale) (fig. 4). It is shown that the phosphorus uptake conformed very closely to the formation of dry matter up to the sixth week, or about the time flowers became visible. This uptake phase was followed by an actual net loss of phosphorus from the plants; this loss was

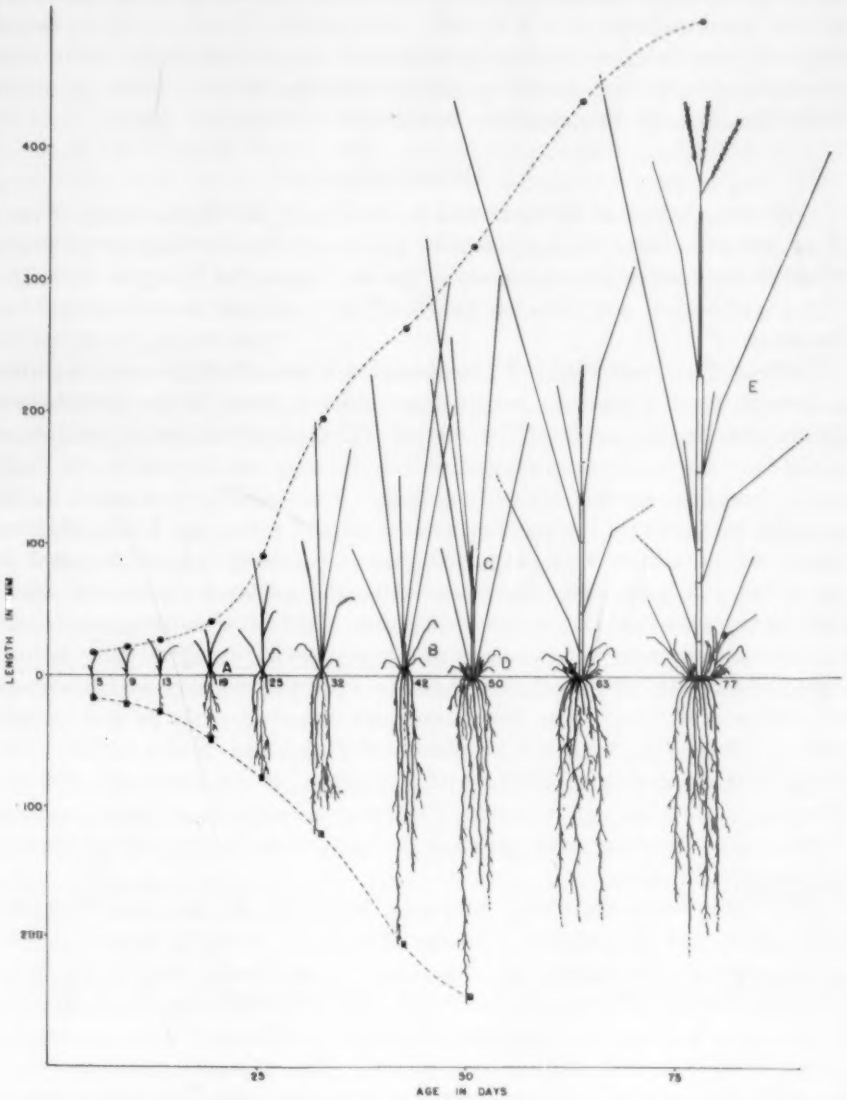

FIG. 5. A semi-diagrammatic illustration of shoot and root elongation and gross morphological development of *Andropogon ischaemum*. The letters refer to: A—appearance of adventitious roots; B—beginning of rapid internode elongation; C—appearance of inflorescences; D—tillering; E—branching.

succeeded by net absorption, though at a relatively slower rate. Figure 5 is a semi-diagrammatic presentation of certain morphological developments in relation to the growth of the shoots and the roots in terms of elongation. By comparing the phosphorus uptake graphs with this figure it is possible to correlate phosphorus uptake with the morphological developments.

Although the plants flowered under the greenhouse conditions, they failed to set seed. According to W. V. Brown (personal communication) meiotic abnormalities were involved. The possible physiological causes of these abnormalities are being investigated. Since this study dealt with vegetative growth and flowering rather than the fruiting phase, it is not likely that meiotic abnormalities would have affected the results.

Discussion

The morphological changes which are a part of development of any plant are reflections of physiological changes. The developmental study reported here attempts to correlate the morphological changes with specific physiological activities on one hand and general overall growth on the other.

Briggs, Kidd, and West (**6**) recalculated Kreusler's data on corn grown in four successive years to obtain a generalized curve of the growth rate throughout the life cycle of the plants. This curve showed a continuous initial increase to a maximum and then a decrease interrupted by two subsidiary maxima on the descending limb. The subsidiary maxima corresponded, respectively, to the appearance of the male and female flowers. Biddulph and Brown (**4**) reported that throughout the development of the cotton plant, the daily increment of both water and dry matter exhibited three distinct maxima, occurring nine days after microsporogenesis, 11 days after megasporogenesis and 16 days after fertilization. In our experiments with *Andropogon ischaemum*, the growth increments showed two distinct maxima. The first maximum occurred early in the growth cycle in association with the beginning of elongation of the leaves. The second maximum was associated with elongation of the internodes, the appearance of flowers and tillering. Further correlation of these maxima with specific developmental events is the subject of further morphological and anatomical study.

The phosphorus-absorbing efficiency, in terms of dry weight of the whole plant and that of the root, also showed two distinct maxima in the eleven-week growth period (figs. 2 and 3). These results may be compared with those of Biddulph and Brown (**4**) who reported that the increment of labelled phosphate was slightly influenced by the developmental events, although well-marked maxima were absent. The decreasing increments of phosphorus uptake in the later growth stages agree with the observations of Williams (**19**) on oats.

Our data show flowering to be associated with a slower rate of growth and of phosphorus uptake. This is in agreement with Bakhuyzen's (**3**)

observations on wheat, although the *A. ischaemum* plants tillered while flowering instead of tillering before flowering as does wheat.

It has already been noted that actual losses of minerals at the time of flowering has been reported by BAKHUYZEN (**2**) and earlier workers. The mechanism involved in this process is not clear. CHIBNALL (**8**) has reported the loss of nitrogen in the form of glutamine excreted by guttation, but there have been no suggestions as to how other elements are lost from plants.

The close parallelism between mineral uptake and increase in dry weight during the early stages of growth is a common observation for many plants (BURD, **7**). The data of this experiment show that the shoot/root ratios decreased gradually as development proceeded, and that a relatively larger proportion of phosphorus was present in the root at the later stages. This is probably of considerable importance in view of the fact that *Andropogon ischaemum* is a perennial grass. As old tillers die off, young tillers continue to develop. The young growing tillers presumably draw part of their food material from the old degenerating tillers, with the root system functioning as a "storehouse."

In the present study, the plants were started from seeds. How plants would behave as to phosphorus absorption when they are started from root stocks or "sods" is not known. However, it is likely that they would follow the same general pattern of phosphorus absorption as the plants started from seeds. The facts which are set forth here suggest further investigation of this pattern of phosphorus absorption as means of indicating somewhat more specifically just when phosphate-containing fertilizers should be applied to provide for their most efficient utilization. There is clearly a suggestion that the level of phosphorus available to the plant during the early stages of development is of more critical importance than the level of phosphorus available to the plant during later developmental stages.

Summary

1. The growth and phosphorus absorption in *Andropogon ischaemum* L. were studied over a period of 12 weeks extending from germination to flowering. The plants were grown in sand cultures in a greenhouse. Two series of plants were grown, the first one from November to January, the second one from January through March.

2. The weekly growth increment obtained by dividing the weekly dry weight increase by the weight at the beginning of the week, exhibited two maxima, the first occurring in the second to third week in both series, and the second in the fifth to sixth week in series 1 and in the fourth to seventh week in series 2. The first maximum was greater than the second. Morphologically, the first maximum was associated with the elongation of the leaf blades, the second one with elongation of the internodes and first tillering.

3. There was a gradual progressive decrease of the shoot/root ratio in-

dicating that root growth was relatively more rapid in the later stages of development.

4. The phosphorus uptake conformed closely to the increase in dry weight up to six to seven weeks or about the time flowers appeared. At the time of flowering there was an actual net loss of phosphorus from the plant.

5. The phosphorus-absorbing efficiency, calculated by dividing the weekly increase in phosphorus by the total dry weight of the plant or that of the root alone, also showed two distinct maxima occurring in both series in the second to third weeks and fifth to sixth weeks. In both series the two phosphorus-absorbing efficiency maxima were approximately of the same order of magnitude.

6. The percentage of phosphorus in the plant was highest during the early germination stages and then went down gradually except when at about the sixth week a second high point was reached. The phosphorus content in the shoot was about one and a half to two times as high as in the root on a unit dry weight basis. The apical portion of the root contained more phosphorus than the basal portion. Young vegetative tillers were higher in phosphorus than old flowering shoots. Relatively more phosphorus was present in the root than in the shoot at the later stages of development as compared with the earlier stages.

Seed of *Andropogon ischaemum* L. used in this experiment were kindly furnished by the San Antonio Nursery of the Soil Conservation Service of the United States Department of Agriculture. This work is part of a project to which several individuals and corporate groups have lent support. It is a pleasure to acknowledge support from the following: Mr. E. W. Brown, Jr.; Mr. Joe Bridwell; Mr. J. Catto, Jr.; Mr. Bryant Edwards; Mr. Ewing Halsell; Mr. L. B. Hapgood; Mr. Richard King; Mr. Tom Lasater; Mr. M. H. W. Ritchie; Mr. G. R. White; Mrs. Anne Burnett Windfohr; Humble Oil & Refining Company; King Ranch; San Antonio Loan & Trust Company; Sawyer Cattle Company; and Swift & Company.

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SULPHUR DIOXIDE FUMIGATION OF WHEAT WITH SPECIAL REFERENCE TO ITS EFFECT ON YIELD

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(WITH SIX FIGURES)

Received April 7, 1950

Introduction

Early investigators of the effect of sulphur dioxide from industrial plants on crops theorized that the leaves of a plant represented its manufacturing power. They reasoned that each plant type was endowed with a standard number of leaves with which to produce a crop and if this standard was reduced by the action of SO_2 the production would be reduced proportionately. This theory was known as the "measured basis for damage computation." Experimentation was necessary to prove or disprove the theory. This is the purpose of the experimental work reported herein.

The experiments were conducted over a five-year period at Hereford, in the San Pedro Valley, a smoke free district, 35 miles air line from the smelter at Douglas, Arizona. Early in the work it became evident that the yield loss resulting from the sulphur dioxide treatment varied noticeably with the weather in different years. Since experimental work dealing with growing plants in the field is subject to many variable factors which are beyond the control of the investigator, many repetitions were necessary. In all, 278 separate tests were made. After treating such crops as beans, potatoes, peppers, onions, wheat, etc. for several years, it became apparent that loss of yield differed with each crop. Vegetative, fructifying and root crops react somewhat differently and there was even certain evidence that varieties within the species were differently affected. Evidently each plant type should be considered separately.

The principal effect of sulphur dioxide on wheat is on the leaves and leaf sheaths because the stomata are most numerous in these organs. In certain very severe treatments however, the glume, awn and even the green stem above the terminal leaf showed the injury. The effect on the yield of marketable produce is a direct result of foliage reduction. Plants may be affected in any stage of growth even before flowering or fruit formation has started. To determine the effect of defoliation caused by SO_2 on the quality and quantity of the produce, the authors devised a method of measuring the per cent. of foliage destroyed. By use of this method and the resultant effect on the yield, as shown in the accompanying graphs, it is possible to predict with reasonable accuracy the per cent. of yield reduction which will follow any given per cent. of foliage reduction.

Historical review

Previous work of this type is lacking, either in published works or in manuscripts in the hands of educational institutions, government agencies or investigators employed in private industry.

The earliest work in this country tending to shed light on the effect of sulphur dioxide on grain was conducted by a Commission whose findings were published in the Selby Report (**1**). Apparatus was devised whereby plants could be treated with the gas under field conditions, but the experimental emphasis was solely on determining the susceptibility factor of certain species, including barley, and the resultant effect on the leaves.

Somewhat later P. J. O'Gara of the American Smelting and Refining Company conducted field experiments, on a limited scale, with wheat, barley and oats. His experiments (unpublished) supplied quantitative information regarding the necessary SO_2 concentrations, time factor, humidity, heat and light values necessary to produce injury to the leaves. O'Gara also recorded resultant yields, but only evaluated the severity of injury to the leaves in such general terms as light, medium and heavy.

M. D. Thomas and George R. Hill, also of the American Smelting and Refining Company, developed apparatus designed to measure continuously and automatically the carbon dioxide exchange and transpiration of plants growing under normal conditions and when treated experimentally with sulphur dioxide. Their experiments covering alfalfa and wheat are published (**2, 3**).

In 1932, Robert E. Swain and Arthur B. Johnson conducted experiments at Stanford University, using wheat plants grown in nutrient solutions in two cabinets under duplicate conditions of temperature, light intensity, humidity and circulating air volume. Sulphur dioxide in regulated amounts was added daily to the air stream passing through one of the cabinets for periods up to six hours each day in such small concentrations that there was no visible effect on the leaves. They found that no reduction in yield occurred unless there was visible foliar injury (**4**).

Between 1927 and 1937 the Canadian National Research Council performed experiments to show the effect on the chemical composition of wheat plants when treated with sulphur dioxide (**5**).

General experimental procedure

SIZE AND ARRANGEMENT OF PLOTS.—The plots were six feet square and arranged as shown in figure 1. The letter "F" indicates fumigated plots and "C" check plots. The ten-foot aisles and two-foot spaces furnish working room. The purpose of this arrangement was to equalize possible irregularities in the soil, cultivation and irrigation. Furthermore, each fumigated plot, with the exception of those at the ends of the rows, had three adjacent check plots, any one of which, or the average of three, could be used as a standard for determining the effect of fumigation. Figure 2 shows the wheat plots in the year 1941.

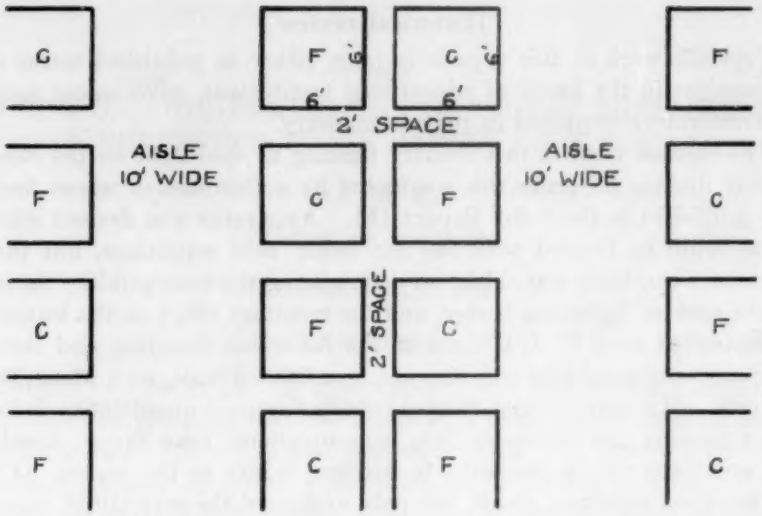

FIG. 1. Planting arrangement of the wheat plots to equalize possible irregularities in soil, cultivation and irrigation. The letter F indicates fumigated plots and C the check or untreated plots.

FUMIGATION EQUIPMENT.—The apparatus used was substantially the same as that developed by P. J. O'Gara. Briefly, it was composed of a closed cabinet of light wood framework covered on sides and top with transparent cellulose acetate sheets. The cabinet was four and one-half feet tall and covered a ground surface of six feet square. The delivery pipe was located at the center top of the cabinet with baffles to distribute the air stream uniformly over the plants within the cabinet. The exit stream was expelled into the ten-foot aisle through holes under the baseboard. To secure the proper air delivery into the cabinet, an electric blower was used having a capacity of 10,000 liters of air per minute when operated on 110-volt direct current. Sulphur dioxide gas was delivered into this air stream in known quantities by means of a calibrated capillary flow-meter.

Two identical cabinets were used and most of the fumigations were made on a half-hourly, instead of an hourly basis as was the practice in most of the O'Gara experiments. In this way a larger number of tests could be made during suitable weather.

APPEARANCE OF SULPHUR DIOXIDE INJURY ON WHEAT FOLIAGE.—There are two types of injury shown in figure 3, acute and chlorotic. The pattern of the injury is difficult to describe and is best indicated by a study of the illustration. Acute injury is most common. The rate at which the affected areas of the leaves dry to a light tan color depends on the humidity of the air. Generally the desiccation becomes complete within two to four days. At this stage the markings can readily be recognized by persons experienced in SO_2 injury diagnosis. Frequently the area immediately adjacent to the midrib is more resistant to the gas action and remains un-

FIG. 2. Wheat plots grown in the year 1941. Plots were six feet square and contained 25 plants each. Half were later treated with sulphur dioxide and half served as checks.

injured. When the dosage is heavy or long continued, the whole leaf blade may be destroyed. Banding occurs from contact with nearby leaves which form a protective covering over a portion of the leaf. If the dosage is mild, a period of four to six days may elapse before the injury manifests itself as a yellowing of the affected areas due to the loss of chlorophyll. This is known as chlorotic injury and is different in appearance from the acute type which is caused by the breakdown and collapse of the leaf cells. The fully developed, highly functional leaves are the first to show injury, and the young, undeveloped blades are quite resistant.

PRELIMINARY TESTS.—The first year that wheat was grown, experimental procedures were standardized, pure varieties developed and methods of determining the per cent. of foliage destroyed were devised. The best locally grown seed available of two varieties of wheat, Baart and Sonora, was used and 32 plots of each variety were planted in the arrangement shown in figure 1. Seed was drilled in rows and later the plants were thinned to exactly 25 plants per plot, allowing each plant one square foot of ground on

FIG. 3. Illustrating the pattern of sulphur dioxide marking on wheat leaves. (1) Banding due to contact with nearby leaves. (3) Typical acute markings. (4) Typical chlorotic margins. (2, 5, 6) Other manifestations.

which to grow. At the season's end, 6.6% of the plants were discarded because they were not true to type. Seed was selected from plants of perfect Baart and Sonora type for planting in subsequent years.

A number of irregularities appeared the first year. Counting the number of leaves destroyed by the treatments and expressing them as a per cent. of the total number produced on the check plants proved unsatisfactory because of the extreme variation in leaf size. Furthermore, wheat blades may elongate and continue to increase in size after being injured by the gas. For example, at time of treatment one-half the area of a leaf might be destroyed but four days later, because of subsequent growth, only one-tenth of the leaf was destroyed. This reduction of area, when expressed as part of the whole continued to decrease until the leaf became full grown. Obviously it was necessary to obtain the leaf area rather than the number of leaves destroyed by the treatment. The average mature leaf which developed each week on the check plants was traced on cross section paper and the area destroyed, in square centimeters, was expressed as a per cent. of the total area of this leaf. This entailed a determination of the number of square centimeters of leaf tissue produced on the untreated

plants each year, because the leaf area of normal plants varied from an average of 4703 sq. cm. per plant in 1941 to 2662 sq. cm. in 1944.

It further became apparent that when plants received several very severe treatments they produced fewer leaves during the season than did the checks. Wheat plants treated once a week throughout their entire growth period, judging by visual observation, appeared to have 90% of the green tissue destroyed. Actual measurements of the foliage area destroyed, when expressed as a per cent. of the foliage area of untreated plants, often gave a figure of no more than 30%. In order to correct this difficulty it became necessary to determine, not only the area destroyed by treatment, but also the area inhibited. To a combination of these two values the term "area lost by treatment" is used in this paper. It was impossible to measure the total leaf area produced on every treated plant (a total of 3350 plants were grown in 1944). However, it was possible to count the num-

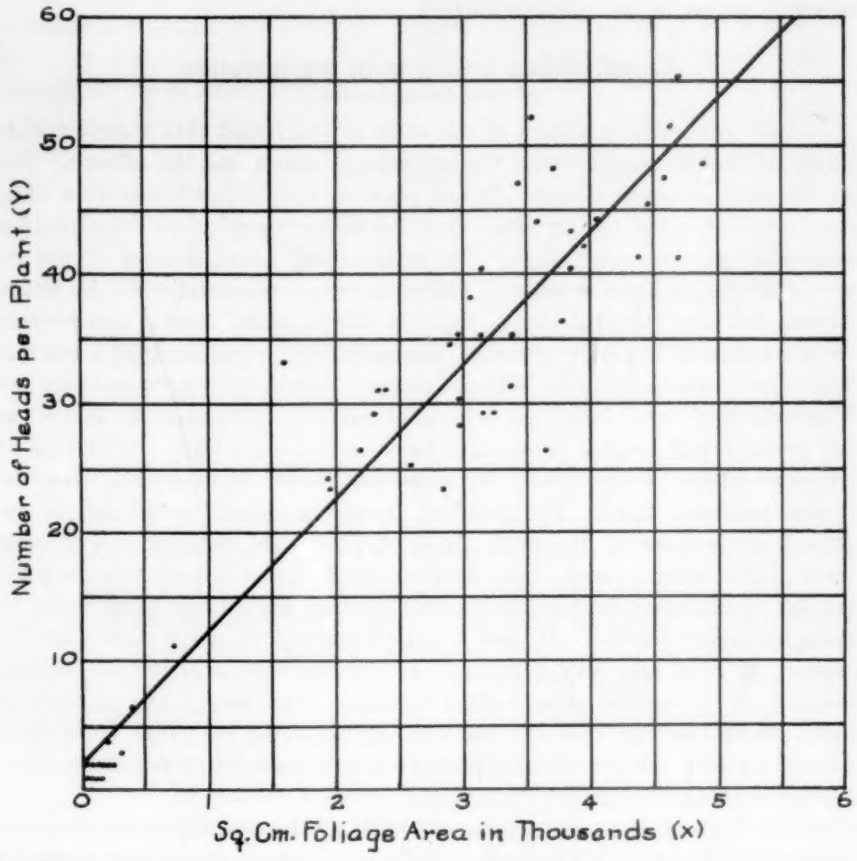

FIG. 4. Correlation between the number of heads and the foliage area of wheat plants. Individual tests, numbering 51, were made during a three-year period. The correlation coefficient has a value of +0.953.

ber of heads produced on each plant at the time of harvest. Because of the growth habit of wheat plant the foliage area is predetermined.

Figure 4 shows a graph based on measurements of the total leaf area of 51 individual, untreated plants made during a three-year period. It will be noted that the greater the number of heads per plant the larger the foliage area per head. The correlation coefficient of the graph, $r = +0.953$ is high and by use of the null hypothesis a highly significant correlation was shown between the leaf areas and the number of heads in the 51 individual determinations, involving plants having from 1 to 55 heads each. The graph may be used with a high degree of accuracy to determine the average leaf area per plant when the number of heads per plant is known.

The difference between the total leaf area of each fumigated plant (as determined by the area-head graph, figure 4) and the leaf area of the average check plant gives the area inhibited because of treatment. To arrive at the total area lost by treatment this figure is added to the measured area actually destroyed by sulphur dioxide.

Complications arising from the operation of natural agencies

In the preliminary phases of the work it was found that a rather wide range of factors entered into the attempt to determine the effect of SO_2 on the yield of wheat plants. If one plant or even a few heads were missing from either the test or check plot the reduction in yield constituted a noticeable experimental error. The outstanding plant disease of the region, affecting wheat, is smut. This was completely controlled by disinfecting the seed the first year. Injuries due to birds, insects and rodents were combatted by every possible means before severe damage occurred. The injury caused by winds was not equally distributed and plants set out where meadow larks killed an occasional seedling did not grow as well as the undisturbed plants. They did, however, serve to limit the growth of adjacent plants which would have become larger than normal had the spaces not been filled. The yield of these transplants could not be included in the yield of the plot. Instead, they were assigned the average yield of the normal plants. An area of wheat seeded adjacent to the plots tended to minimize the bird and insect damage until these pests could be brought under control. It was found necessary to drive stakes at the corners of each plot and surround the growth with a string to prevent lodging of the border plants after heading. The larger the number of plots allotted to any test, the smaller was the error resulting from these adverse factors. It seemed desirable to use at least 50 plots before drawing definite conclusions.

Table I shows the variation in the yield of the check plots in the four years since the preliminary work. Probably much of the yearly variation in yield was due to weather. In the year of lowest yield there was a correspondingly low wheat production on farms growing that crop.

TABLE I

YEARLY VARIATION IN YIELD OF CHECK PLOTS

YEAR	NUMBER OF PLOTS	YIELD IN OUNCES	
		TOTAL	AVERAGE
1941	36	1980.19	55.10
1942	35	1680.00	48.00
1943	41	1407.79	34.34
1944	134	5503.24	41.07

Plan of fumigation treatments

In carrying out the tests an attempt was made to subject the experimental plants to treatments such as may be received under actual field conditions on farms near smelters. We have tried to attain this end, on the one hand by giving some plants single treatments with different concentrations of sulphur dioxide at various stages of plant growth, and on the other hand, by giving others repeated treatments at many stages throughout the growing period.

Table II shows the year in which the tests were made, the number of

TABLE II

NUMBER OF PLOTS TREATED

YEAR	ONE FUMIGATION			TWO FUMIGATIONS	THREE FUMIGATIONS	MULTIPLE FUMIGATIONS	
	EARLY STAGE	BLOOM STAGE	LATE STAGE	BLOOM STAGE LATE STAGE	EARLY STAGE BLOOM STAGE LATE STAGE	WEEKLY	BI-MONTHLY
1940	9	9	9	1	1	1	2
1941	.....	12	.....	12	12	.....	.....
1942	.....	.....	.....	14	15	4	2
1943	.....	.....	.....	19	18	2	2
1944	.....	130	.....	.....	.....	2	2

plots treated, the stage of treatment and the frequency of fumigations. It will be noted that in the year 1944, 130 plots were fumigated once. In order to subject the plants to treatments in every stage of their growth, 10 plots were treated each week for 13 weeks. This covered the entire span of the growth period, from the time the plants had made two blades until ripening had begun.

Correlation between foliage area destroyed by SO_2 (a measure of leaves destroyed) and yield

SINGLE FUMIGATIONS.—In 1944 the experimental emphasis was on single fumigations. In that year 10 plots were treated each week for 13 weeks, 130 plots in all. In this way plants were treated in every stage of growth from the time they had only two leaves until they were nearly mature.

The only other single fumigations used in making the graph, figure 5, were made in 1941 when 12 plots were treated in the bloom stage. The single fumigations made in 1940 could not be used because the method of leaf area measurement had not been fully developed and standardized until the year 1941.

The leaf area destroyed by the treatments was determined in square centimeters for each of the 25 plants in every plot and the average number of square centimeters destroyed per plant was computed. The normal foliage area was determined on eleven average plants from the check plots

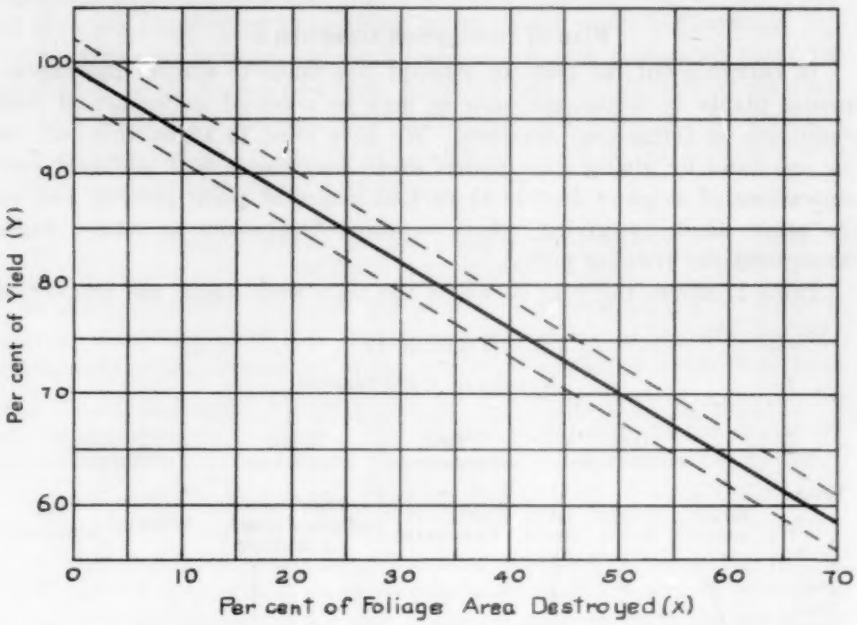

FIG. 5. Correlation between the per cent. of foliage area destroyed and the per cent. of yield when 142 wheat plots were fumigated once during growth. The formula for the regression line is $Y = 99.58 - .558X$. The standard deviation is 2.694 and the correlation coefficient + 0.928. The result of each test is given in table III.

and the per cent. of area destroyed for each of the 142 fumigated plots was determined. When the wheat was fully matured, the plots were harvested and threshed separately and the per cent. of yield computed, using the check plot as the standard. With these two variables, per cent. of area destroyed X, and per cent. of yield Y, the regression line shown in figure 5 was calculated by using the method of least squares. The equation for this regression line is $Y = 99.58 - .588X$.

The large number of tests and the overlapping of the points in the region where Y = 98% to 101% and X = 0% to 3% made it impractical to spot each of the individual 142 determinations on the graph. The figures, however, are supplied in detail in table III.

The standard deviation (Sy) of the individual plot yields from the regression line is 2.69%. When the independent variate (per cent. of area destroyed) is known the dependent variate (per cent. of yield) may be determined from the graph within the limits of the standard deviation band.

In order to determine the weight that may be attached to this experimental evidence, the correlation coefficient (r) was determined. This

TABLE III

SINGLE FUMIGATIONS OF WHEAT

Year 1941 Yield in ounces					Year 1944 Yield in ounces				
Plot no.	Fumigated	Check	% Area destroyed	% of crop	Plot no.	Fumigated	Check	% Area destroyed	% of crop
279	45.27	52.71	32.0	85.89	113	35.25	35.15	.78	100.28
280	51.15	55.75	30.7	91.75	114	37.81	38.00	.46	99.50
281	50.23	54.88	22.6	91.53	115	39.16	39.21	.11	99.87
282	45.63	51.45	24.5	88.69	116	35.02	33.76	.02	103.73
283	50.72	55.43	24.9	91.50	66	39.50	40.00	1.11	98.75
284	50.47	55.57	28.8	90.82	67	40.15	41.00	1.69	97.93
285	53.41	55.15	13.2	96.84	68	37.25	37.78	1.40	98.60
286	47.34	51.63	21.2	91.69	69	39.25	40.20	2.06	97.64
287	49.05	53.70	15.2	91.34	101	28.25	31.45	1.20	89.83
288	44.53	50.25	12.7	88.62	102	34.75	33.81	1.24	102.78
289	49.73	52.01	10.7	95.62	103	31.69	32.00	.66	99.03
290	46.56	50.96	12.9	91.37	104	35.77	36.61	1.61	97.71
Average			20.73	91.34	105	36.72	37.75	2.41	97.27
					106	42.25	42.25	.00	100.00
					70	40.75	41.00	.00	99.39
					71	42.62	42.89	.23	99.37
					72	44.25	44.25	.78	100.00
					73	49.50	48.50	.59	102.06
					74	46.25	46.75	4.33	98.93
					75	42.25	43.00	1.76	98.26
					76	40.53	40.75	.13	99.46
					77	47.33	46.79	2.51	101.15
					78	41.51	42.00	2.29	98.83
					79	39.48	40.75	3.35	96.88
					80	44.75	44.50	.00	100.56
					81	42.50	42.67	.33	99.60
					82	41.85	43.00	3.12	97.33
					83	42.00	44.00	5.46	95.45
					84	44.00	44.35	2.19	99.21
					85	38.22	39.61	4.34	96.49
					86	43.25	44.25	3.54	97.74
					87	45.25	46.25	6.32	97.84
					88	41.21	43.50	6.26	94.74
					89	37.35	39.25	6.40	95.16
					90	41.19	41.32	.00	99.69
					91	40.11	40.28	.00	99.58
					92	42.83	44.24	4.78	96.81
					93	38.82	41.45	7.96	93.66
					94	39.87	43.75	10.71	91.13
					95	31.45	33.21	11.36	94.70
					96	40.06	43.25	9.08	92.62
					97	33.22	36.50	10.73	91.01
					98	36.50	40.25	11.49	90.68
					99	32.78	37.80	17.46	86.72
					56	35.36	41.37	18.89	85.47

Year 1944 Yield in ounces				
Plot no.	Fumigated	Check	% Area destroyed	% of crop
127	40.25	40.47	.03	99.46
128	39.75	39.75	.08	100.00
129	35.77	35.85	.14	99.78
130	33.15	32.00	.11	103.59
131	36.55	36.66	.02	99.70
132	34.38	33.00	.04	104.18
133	40.00	40.25	.00	99.38
134	36.00	36.50	.00	98.63
135	38.44	38.75	.00	99.20
136	41.01	41.25	.00	99.42
117	39.20	39.50	.22	99.24
118	38.06	38.08	.16	99.95
119	45.25	44.75	.13	101.12
120	38.76	39.00	.11	99.38
121	38.42	38.92	.11	98.72
122	40.25	40.50	.07	99.38
123	39.51	39.66	.10	99.62
124	39.75	39.75	.02	100.00
125	42.31	42.25	.00	100.14
126	43.00	43.25	.00	99.42
107	39.24	39.75	1.54	98.72
108	39.75	40.25	1.06	98.76
109	35.15	35.00	.88	100.43
110	34.25	33.50	.88	102.24
111	35.35	35.50	.89	99.58
112	35.76	36.38	.54	98.30

TABLE III (*Continued*)

SINGLE FUMIGATIONS OF WHEAT

Year 1941 Yield in ounces					Year 1944 Yield in ounces				
Plot no.	Fumigated	Check	% Area destroyed	% of crop	Plot no.	Fumigated	Check	% Area destroyed	% of crop
57	36.50	44.62	24.55	81.80	4	39.00	39.55	1.57	98.61
58	36.23	42.00	18.32	86.26	5	35.75	36.25	.29	98.62
59	42.54	50.75	19.30	83.82	6	36.75	36.68	.76	100.19
60	38.75	42.50	11.63	91.18	7	41.75	42.00	.39	99.40
61	40.75	44.75	12.43	91.06	8	41.25	41.50	.63	99.40
62	40.25	42.50	6.95	94.71	9	38.78	39.00	.84	99.44
63	41.50	44.01	7.67	94.30	10	38.00	38.49	.40	98.73
64	39.78	39.78	.00	100.00	11	42.75	43.50	.98	98.28
65	38.75	39.50	1.80	98.10	12	44.50	45.50	4.00	97.80
46	38.00	42.50	13.92	89.41	13	47.75	46.75	.00	102.14
47	46.00	45.75	.87	100.55	14	45.75	46.00	3.80	99.46
48	41.50	52.50	22.23	79.05	15	41.75	43.99	5.89	94.91
49	44.50	45.00	3.38	98.89	16	41.25	43.50	5.94	94.83
50	41.25	42.53	4.06	96.99	17	40.75	43.75	8.04	93.14
51	40.14	41.50	4.12	96.72	18	36.50	38.75	6.62	94.19
52	38.54	39.24	2.25	98.22	19	42.25	47.25	18.05	90.48
53	35.25	34.50	.21	102.17	20	35.75	39.41	11.87	90.71
54	28.05	30.75	.05	91.22	21	37.75	41.99	12.47	89.90
55	40.95	41.07	.00	99.71	22	41.50	48.25	20.53	86.01
1	35.38	42.25	21.87	83.74	23	39.25	39.25	.00	100.00
2	37.75	44.05	19.19	85.70	24	39.25	40.38	2.90	97.20
33	31.00	46.00	43.99	67.39	25	36.50	38.52	6.37	94.76
34	32.50	32.81	5.30	99.06	26	35.50	36.75	5.83	96.60
35	33.00	41.25	25.77	80.00	27	36.50	38.50	6.27	94.81
36	33.09	37.90	16.88	87.31	28	38.00	40.75	7.97	93.25
37	38.00	44.06	18.69	86.25	29	34.78	37.75	9.07	92.13
38	41.00	43.95	8.12	93.29	30	36.75	42.00	16.63	87.50
39	46.50	47.25	5.90	98.41	31	39.75	55.25	60.05	71.95
40	45.75	45.00	.00	101.67	32	29.75	54.00	66.91	55.09
3	37.25	37.38	.04	99.65		Average		6.02	95.32

In the year 1944 the first 10 plots (127 to 136, inclusive) were fumigated in the first week of growth, the next 10 plots in the second week of growth, this system being continued up to 13 weeks.

measures the degree to which the average relationship holds in practice. In this case the value of r is + 0.928 which is beyond the 1% level of significance, calculated by the null hypothesis. This high value of r indicates that the correlation between the individual tests is highly significant.

The data were further correlated using the variates per cent. of area lost (area destroyed plus area inhibited) already defined on page 671, and per cent. of yield. In this case the regression line equation became $Y = 101.40 - .60X$. This line is nearly parallel to the regression line shown in figure 5 and the origin of the line on the Y axis would be only 1.82% higher. Thus it is shown that with single fumigations the area inhibited is not particularly important. Statistically, however, it serves to explain why the origin of the regression line, figure 5, falls below 100% of crop.

Of the total foliage which a wheat plant develops during its life, no more than 60% is green and functioning at any stage. Many of the plots

showed as high as 95% destruction of the foliage present at the time of treatment, but when expressed in per cent. of the total foliage area made by the plant during the season, this generally fell below 30%.

In 1941 the 12 plots fumigated once in the bloom stage were insufficient in number to warrant any very definite conclusion, yet it is interesting to note by the average given in table III that there was less crop damage for each per cent. of leaf area destroyed than in the tests made during the year 1944. In 1944 the check plants averaged only 2662 sq. cm. foliage area while in 1941, a year of high yield, the average plant made 4703 sq. cm. Thus it is tentatively assumed that the more thrifty the crop the less damage is sustained by the action of SO_2.

Summation of the tests given in table III by groups of various stages of plant growth at the time of fumigation showed no distinct trend that would indicate a critical stage at which the yield might be more severely affected. At the two extremes of the growth period there may be some evidence of a change in the amount of damage for each per cent. of area destroyed, a slight increase at the earliest stage of growth and a decrease when the plants are near maturity. This trend is not at all conclusive as it falls well within the standard deviation band.

Correlation between foliage area lost (area destroyed plus area inhibited) and yield

DOUBLE AND TRIPLE FUMIGATIONS.—During the years 1941 to 1943, inclusive, wheat in 45 plots was fumigated twice. The first treatment was at the male anthesis after the fertile tillers had expanded the terminal blades and the second treatment when the plants were nearing maturity. An additional 45 plots were treated three times. The first treatment was in the vegetative stage, the second at male anthesis and the third in the early dough stage before the plant showed signs of maturity.

When wheat plants are treated more than once during growth, the leaf area inhibited by the treatments is important; therefore, to arrive at the foliage area lost because of the treatment it is necessary to add the area inhibited to the area destroyed and to compare this total with the average foliage area of the check plants to determine the per cent. of foliage area lost.

The area destroyed was determined in the same manner as already explained under single fumigations, but in order to arrive at the area inhibited it was necessary to count the number of heads made by both the fumigated and check plants at the time of harvest. This has been fully discussed under the heading Preliminary Tests. Because the area inhibited was determined by using the average leaf area produced on representative check plants, it was necessary to express the independent variate (area lost) as a deviation from the mean as shown in figure 6. It seems somewhat ambiguous to indicate a minus per cent. of area lost, but it should be remembered that this is simply the result of an average figure being used

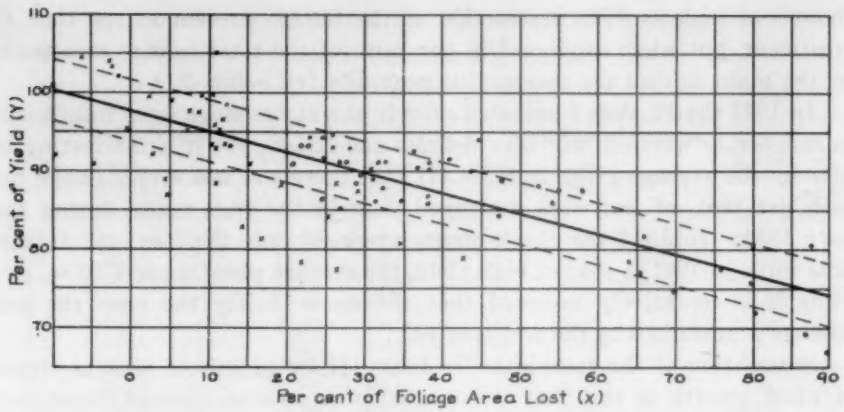

FIG. 6. Correlation between the per cent. of foliage area lost (area destroyed plus area inhibited) and the per cent. of yield when 90 wheat plots were fumigated with sulphur dioxide two or three times during growth. The formula for the regression line is $Y = 97.617 - .263X$. The standard deviation is 4.05 and the correlation coefficient is X0.843.

Legend—(●) Tests made during 1941. (o) Tests made during 1942. (x) Tests made during 1943.

as a basis for computing percentages. Because of individual plant variations, there were eight plots out of the 90 which had unusual thrift and vigor, so that even though there was a reduction of foliage due to treatment these eight plots still produced a larger foliage area than the average check plot.

The result of treating each plot is spotted on the graph, figure 6, using different kinds of points, as indicated in the legend, for the years in which the respective treatments were made. The equation for the regression line as worked out by the method of least squares is $Y = 97.62 - .26X$ and the standard deviation is 4.05. The high value of the correlation coefficient (+0.843) indicates that the correlation between the individual tests is highly significant. The origin of the regression line at 97.62% of yield instead of 100% is not significant as it falls well within the standard deviation band. However, in searching for an explanation for this occurrence, the following point seems worthy of consideration. In the process of determining the number of square centimeters of foliage area destroyed, it was necessary to handle every leaf of the fumigated plants after each treatment. Experience has shown that handling alone may damage the plants and lower the yield. The check plants were not exposed to this type of treatment, therefore this constitutes an experimental error which we were unable to eliminate even by extremely careful work. Very probably this error would amount to at least the 2.38% necessary to place the origin of the regression line at 100% of yield.

Observing the tests indicated by years on the graph, it will be noted that the greatest loss of yield, per per cent. of area lost, was in the year

1943, and the least in 1941. Comparing this with the average foliage area (a measure of thrift) produced by the check plants in these same years,

4703 sq. cm. in 1941
3951 " " " 1942
3642 " " " 1943

we observe a distinct correlation between thrift of plants and damage to yield from sulphur dioxide treatment.

A further comparison can be made between the regression line for single treatments and the regression line for double and triple treatments. Comparing the graphs in figures 5 and 6 it is noted that the slope of the line, which indicates the degree of damage, is greater in the case of single fumigations. It is a reasonable assumption that at least part of this difference is due to the recovery of plants between treatments. It is a greater shock to the plant's vital processes to remove 30% of its foliage at one treatment than to remove 15% at two different treatments, allowing several weeks between for recovery. Another probable reason for the difference in slope is due to the fact that no double and triple fumigations were made during 1944, the year of poorest plant growth when the average

TABLE IV

MULTIPLE FUMIGATIONS OF WHEAT

	YEAR 1942			
	YIELD IN OUNCES			
PLOT NO.	FUMIGATED	CHECK	% AREA LOST	% OF CROP
152	31.25	48.00	97.6	65.1
153	31.00	47.00	93.8	66.0
154	22.00	42.00	99.0	52.4
155	24.00	43.00	94.7	55.8
156	33.30	51.00	95.2	65.3
157	32.00	48.00	96.1	66.7
	YEAR 1943			
	YIELD IN OUNCES			
PLOT NO.	FUMIGATED	CHECK	% AREA LOST	% OF CROP
352	32.01	36.07	26.6	88.7
353	33.63	42.70	34.8	78.8
354	19.07	23.48	43.0	81.2
355	19.82	29.49	43.8	67.2
	YEAR 1944			
	YIELD IN OUNCES			
PLOT NO.	FUMIGATED	CHECK	% AREA LOST	% OF CROP
41	34.50	42.50	27.8	81.2
42	38.25	46.75	23.1	81.8
43	33.47	47.25	40.4	70.8
45	33.75	50.75	43.4	66.5

foliage area of the check plants was only 2662 sq. cm. In that year the emphasis was on single fumigations, the double and triple fumigations being omitted.

MULTIPLE FUMIGATIONS.—During the years 1942 to 1944 inclusive, eight wheat plots were treated once a week from the time the seedlings had made three leaves until the plants were nearly mature, thus making a total of 13 weekly treatments. Every leaf that the plants had produced showed at least some degree of damage. Another six plots were treated every two weeks during their growth. The main purpose of these frequent treatments was to discover whether the yield could be reduced to zero. A second purpose was to show that severe fumigations are not to be considered comparable to a blight which prevents fructification or even kills the plants outright. These tests indicate that severe and repeated fumigations resemble severe prunings, in that the plants continue to grow and to develop their fruiting organs, though the crop may be much reduced and may mature ten days late depending somewhat on the severity of the test. For the detail of the resultant effect on the yield of these 14 tests refer to table IV.

DAY VERSUS NIGHT FUMIGATIONS.—A concentration of SO_2 sufficient to damage wheat plants in 30 minutes, viz. 15 p.p.m., was applied to four wheat plots, beginning two and a half hours before sunset, for a period of one hour each. Plants in the first two plots, fumigated in full sunlight were severely damaged. The third plot, fumigated partly in sunshine and partly in the dusk was only mildly affected. The fourth plot, fumigated in very dim light or complete darkness was not marked at all, the plants showing no effect whatever from the treatment.

Summary

Three methods of correlation were used as a basis for determining production loss when wheat was subjected to sulphur dioxide injury, namely, correlation between per cent. of leaves destroyed and yield, per cent. of foliage area destroyed and yield and per cent. of foliage area lost and yield. The first, per cent. of leaves destroyed and yield, has been used in smelter districts since 1923 as a means of determining the amount of damage sustained by a crop. The method was not sufficiently accurate for experimental procedures there being great variation in the size of the leaves produced on individual wheat plants. Obviously the measure of the manufacturing power of the plant was the foliage area, rather than the number of leaves which it produced.

Graphs are given, based on four years' work involving 278 separate tests. These provide a guide whereby the per cent. of yield loss may be determined, within the narrow limits of the standard deviation band, when wheat plants are subjected to: (a) only one treatment with SO_2 gas when the foliage area destroyed is known, and (b) double and triple treatments when the foliage area lost is known.

Tests conducted in years of good plant growth showed distinctly less damage for each per cent. of foliage area destroyed than in years of poor growth. The more thrifty the plants the less damage was occasioned by the SO_2 markings.

In no correlation, involving several tests, did the per cent. of crop loss ever reach the per cent. of foliage area destroyed or the per cent. of foliage area lost. Generally it ranged between .26% and .62% of crop reduction for each 1% foliage reduction, depending on the thrift of the plant and the frequency of treatments.

Wheat plants sustained less damage by having 15% of their foliage destroyed twice during the season, with an interval for recovery, than when 30% of the foliage was destroyed by one treatment.

Wheat treated at night showed no effect from SO_2 concentrations sufficient to produce severe foliage injury in sunlight.

Grateful acknowledgment is made to the following persons: for operation of apparatus and painstaking work in securing and recording data, Charles R. Davis and Walter G. Sackett; for valued suggestions and correlation of experimental evidence, George R. Hill, William Crocker, Moyer D. Thomas, L. V. Olson and P. W. Zimmerman.

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GROWTH RESPONSES OF TOMATO TO NUTRIENT IONS ADSORBED ON A PUMICE SUBSTRATE

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(WITH FIVE FIGURES)

Received April 2, 1949

Introduction

Preliminary research with natural pumice (**18, 23**) has indicated that it might be a useful and inexpensive substrate in experiments on the mineral nutrition of plants. Appreciable surface activity appears to be associated with the high porosity of pumice. An experiment was undertaken to determine the availability to roots of specific elements held on different lots of pumice each of which had been immersed in a single salt solution. This investigation was designed to trace plant growth during the progressive diminution of an individual nutrient element in the substrate, the entire quantity of which was initially supplied in an adsorbed state on the pumice, in the presence of a nutrient solution containing all other essential ions in a soluble form and supplied at a uniform rate.

Methods

The pumice used in this experiment was first leached of its readily soluble components and different lots thereof then soaked in single salt solutions, each containing a different nutrient element as described below. A single lot of natural pumice (table I) consisting of pieces one inch or less in dia-

TABLE I

CHEMICAL ANALYSIS OF PUMICE*

Silica	72.90%	Soda	3.64%
Alumina	11.28	Potash	4.38
Iron oxide	0.86	Sulfuric anhydride	0.03
Titanium oxide	0.06	Loss by ignition	5.20
Calcium oxide	0.80	Organic material content	0.022
Magnesium oxide	0.36	Water soluble content	0.13

* Analysis furnished by Pumice Corporation of America.

meter was first leached of readily soluble material by submersion for 24 hours in a large volume of 0.04N hydrochloric acid solution containing acid in considerable excess of that required to neutralize the alkalinity of the pumice itself (**21**). The pumice was then washed in distilled water until no evidence of chlorine or shift in pH of the eluate could be detected. Three different sublots of this leached pumice were then immersed for 48 hours in 0.1M single salt solutions containing $CaCl_2$, $MgSO_4$ or KCl respectively. Analyses of these salt solutions containing pumice indicated considerable cation ad-

sorption. Equilibrium in distribution of calcium, magnesium and potassium ions was attained between the salt solution and pumice during a 48-hour period of soaking. After soaking, these three sublots of pumice were again transferred to distilled water, thoroughly rinsed and then air dried.

A fourth or phosphate series was similarly prepared except that the natural pumice was initially leached in a 0.1N solution of NaOH and then soaked in an excess of 0.5N solution of H_3PO_4. Four experimental series of 10 jars each of pumice were arranged as follows: the first containing the pumice previously soaked in $CaCl_2$, the second pumice soaked in $MgSO_4$, the third pumice soaked in KCl and the fourth pumice soaked in H_3PO_4. Uniform amounts, 3900 grams dry weight, of treated pumice were placed in three gallon glazed jars and again leached for one week in three liters of distilled water. At the end of this time the final amounts of the various ions still retained in adsorbed form on the pumice were determined (table II). Each pumice-adsorption complex constituted one experimental culture.

TABLE II

TOTAL QUANTITIES OF THE VARIOUS ELEMENTS IN MILLIEQUIVALENTS ADSORBED ON THE PUMICE PER JAR

SERIES	ELEMENT	QUANTITY INITIALLY ADSORBED	QUANTITY PRESENT AFTER PLANTS GROWN ON THE SUBSTRATE
		m.e.	*m.e.*
–Ca	Ca	77.85	122.82
–K	K	60.06	4.23
–Mg	Mg	47.34	2.49
–P	P	84.57	18.09

A nutrient solution (table III) containing all essential elements except one was added to each of the foregoing series. The only available source of

TABLE III

MILLIMOLAR CONCENTRATION OF SALTS IN ORIGINAL NUTRIENT SOLUTION ADDED TO VARIOUS SERIES*

		First to fourth weeks		
Control (gravel)	0.703 KNO_3	0.522 KH_2PO_4	0.591 $MgSO_4$	1.726 $Ca(NO_3)_2$
–K (pumice)	0.699 $NaNO_3$	0.741 NaH_2PO_4	0.591 $MgSO_4$	1.726 $Ca(NO_3)_2$
–Mg (pumice)	0.703 KNO_3	0.522 KH_2PO_4	0.598 Na_2SO_4	1.726 $Ca(NO_3)_2$
–Ca (pumice)	0.703 KNO_3	0.522 KH_2PO_4	0.591 $MgSO_4$	3.496 $NaNO_3$
–P (pumice)	0.703 KNO_3	.....	0.591 $MgSO_4$	1.726 $Ca(NO_3)_2$
		Fifth to twelfth weeks		
Control (gravel)	1.406 KNO_3	1.044 KH_2PO_4	1.182 $MgSO_4$	3.452 $Ca(NO_3)_2$
–K (pumice)	1.398 $NaNO_3$	1.482 NaH_2PO_4	1.182 $MgSO_4$	3.452 $Ca(NO_3)_2$
–Mg (pumice)	1.406 KNO_3	1.044 KH_2PO_4	1.196 Na_2SO_4	3.452 $Ca(NO_3)_2$
–Ca (pumice)	1.406 KNO_3	1.044 KH_2PO_4	1.182 $MgSO_4$	6.992 $NaNO_3$
–P (pumice)	1.406 KNO_3	.....	1.182 $MgSO_4$	3.452 $Ca(NO_3)_2$

* All series received traces of microelement boron, copper, iron, manganese and zinc in nutrient solution.

this missing element was that adsorbed on the pumice. The series depending upon the pumice for their supply of the specific element are designated as –Mg, –K, –Ca and –P respectively. A fifth separate series comprising an inert quartz gravel substrate in which all essential elements were supplied in the nutrient solution in soluble form was also run and designated as the control series. The solutions were all drained weekly and immediately renewed at the ion concentrations indicated (table III). All culture solutions were brought to an initial pH of 6.4 with sodium hydroxide before addition to the jars. During growth of plants, chemical analyses for magnesium, potassium, calcium, nitrogen and phosphorus were made of the residual solutions drained from the jars at the end of each week (table IV).

TABLE IV

SOLUBLE IONS IN PARTS PER MILLION IN RESIDUAL NUTRIENT SOLUTIONS AS DRAINED FROM JARS AT WEEKLY INTERVALS DURING GROWTH OF PLANTS IN EACH SERIES

Weeks	1	2	3	4	5*	6	7	8	9	10	11	12
Series	**Calcium in residual nutrient solution, p.p.m.**											
Control (gravel)	.....	37.6	62.8	58.0	103.2	85.2	65.6	60.0	36.4	16.4	28.6	40.0
–Mg (pumice)	.....	70.4	88.4	54.0	97.6	74.0	15.2	12.8	6.4	5.8	0.0	6.4
–K (pumice)	138.0	100.0	80.0	62.8	60.0	72.8	0.0	12.0	5.8	0.0	10.0	22.0
–Ca (pumice)	636.0	184.0	176.8	56.6	50.4	13.6	11.0	6.4	4.7	4.0	13.4	5.3
–P (pumice)	40.0	27.2	22.0	18.8	40.0	80.0	17.6	19.2	27.0	9.6	12.6	32.0
	Potassium in residual nutrient solution, p.p.m.											
Control (gravel)	.....	40.0	24.8	9.6	24.2	0.0	5.4	0.0	0.0	0.0	0.0	2.0
–Mg (pumice)	.....	55.6	22.8	35.8	45.2	5.0	5.0	0.0	5.0	0.0	0.0	0.0
–K (pumice)	500.0	320.0	169.8	102.4	68.8	25.6	2.4	T†	0.0	2.2	0.0	0.0
–Ca (pumice)	80.0	28.2	45.8	32.6	45.6	40.6	9.6	17.7	41.2	35.2	64.0	27.2
–P (pumice)	12.4	7.6	7.0	6.0	9.0	5.0	0.0	0.0	3.0	0.0	0.0	0.0
	Magnesium in residual nutrient solution, p.p.m.											
Control (gravel)	.....	15.0	21.0	18.0	21.3	9.9	2.1	7.8	9.0	8.4	9.9	11.1
–Mg (pumice)	.....	48.0	61.5	10.2	30.0	13.5	3.0	1.2	1.5	T	T	T
–K (pumice)	14.2	22.8	14.4	12.0	8.1	13.8	1.5	1.2	T	T	2.4	4.8
–Ca (pumice)	15.2	22.5	19.2	14.4	15.3	2.4	1.8	1.8	2.1	3.9	12.3	1.8
–P (pumice)	10.8	12.0	12.0	11.1	14.1	15.0	4.8	2.4	2.4	2.1	3.0	6.9
	Nitrogen in residual nutrient solution, p.p.m.											
Control (gravel)	.....	64.8	61.5	54.5	71.5	31.0	13.6	4.8	1.6	1.8	4.7	5.1
–Mg (pumice)	.....	84.0	75.0	72.0	80.5	56.0	43.0	5.9	1.1	1.4	2.5	4.6
–K (pumice)	83.5	89.0	72.5	66.0	77.5	59.5	37.0	3.1	1.3	2.3	4.2	7.2
–Ca (pumice)	74.0	77.5	76.0	75.0	79.0	71.5	41.5	32.2	80.0	79.0	84.0	72.0
–P (pumice)	56.0	62.0	63.5	61.0	78.5	70.0	48.5	7.8	1.1	1.4	1.7	2.0
	Phosphorus in residual nutrient solution, p.p.m.											
Control (gravel)	.....	15.0	13.0	22.5	25.0	10.5	1.1	T	1.3	T	1.4	1.7
–Mg (pumice)	.....	19.0	18.8	17.5	21.8	19.5	4.3	T	0.0	T	T	1.4
–K (pumice)	22.5	22.5	23.5	46.0	21.8	20.0	1.0	1.2	0.0	T	3.2	1.6
–Ca (pumice)	17.5	16.5	16.8	13.3	23.3	24.3	17.6	23.0	36.5	16.0	27.5	17.5
–P (pumice)	185.3	180.2	107.1	68.0	.....	16.0	3.0	T	1.6	0.0	0.0	0.0

* Concentration of salts in original nutrient solution (Table III) doubled at fifth week and so maintained thereafter.

† T indicates a trace present.

Seeds of tomato, variety Pan America, were germinated in sand at a temperature of approximately 27° C. The sand was kept moist with distilled water, and an occasional application of dilute complete nutrient solution was made to prevent the appearance of deficiency symptoms. On February 9, 24 days after the seeds were sown, plants which had attained a uniform height of 45 millimeters were transferred to the jars of all series. Throughout the period of the experiment, these plants were pruned to one main axis for convenience of handling and measurement. Mean diurnal and nocturnal temperatures were maintained at 29° C and 19° C respectively. A mean relative humidity of 50% was maintained over the period of the experiment. Mean intensity of normal daylight for the months during which the plants were grown was 1250 foot-candles. On overcast days, natural daylight was supplemented with 40-watt fluorescent "daylight" lamps.

TABLE V

ELEMENTAL COMPOSITION OF VARIOUS PLANT PORTIONS AS PERCENTAGE OF DRY WEIGHT AND TOTAL QUANTITY IN MILLIGRAMS AT THE STEM ELONGATION STAGE

Element	Roots		Leaves		Stems	
	%	mg.	%	mg.	%	mg.
			Control Series			
Ca	1.40	0.70	4.24	14.42	3.24	2.27
K	5.08	2.54	4.90	16.66	10.74	7.52
Mg	0.60	0.30	0.76	2.58	0.83	0.58
N	2.78	1.39	5.48	18.63	2.33	1.63
P	0.71	0.36	1.06	3.60	0.63	0.44
			−Mg Series			
Ca	0.64	0.38	2.77	8.03	0.89	0.62
K	4.56	2.74	4.26	12.35	10.48	7.34
Mg	1.00	0.60	1.28	3.71	1.13	0.79
N	2.88	1.73	5.80	16.82	2.30	1.61
P	0.72	0.43	1.08	3.13	0.61	0.43
			−K Series			
Ca	1.06	1.06	3.65	11.68	2.03	1.83
K	5.22	5.22	5.20	16.64	11.46	10.31
Mg	0.62	0.62	0.73	2.34	0.68	0.61
N	2.95	2.95	5.29	16.93	2.16	1.94
P	0.93	0.93	1.05	3.36	0.74	0.67
			−Ca Series			
Ca	1.53	1.38	4.15	13.28	3.02	2.42
K	3.60	3.24	4.52	14.46	10.24	8.19
Mg	0.59	0.53	0.50	1.60	0.66	0.53
N	2.78	2.50	5.36	17.15	2.50	2.00
P	0.89	0.80	1.00	3.20	0.66	0.53
			−P Series			
Ca	0.89	0.62	4.18	9.20	2.56	1.28
K	2.66	1.86	2.12	4.66	6.40	3.20
Mg	0.66	0.46	0.71	1.56	0.78	0.39
N	2.84	1.99	4.85	10.67	2.33	1.17
P	2.54	1.78	1.15	2.53	0.99	0.50

Samples for chemical analysis were taken in the late afternoon at five stages in the developmental cycle, as follows: at (1) stem elongation (table V), (2) flower bud primordia development (table VI), (3) anthesis (table VII), (4) early fruit enlargement (table VIII), and (5) the mid-fruiting

TABLE VI

ELEMENTAL COMPOSITION OF VARIOUS PLANT PORTIONS AS PERCENTAGE OF DRY WEIGHT AND TOTAL QUANTITY IN MILLIGRAMS AT THE FLOWER BUD PRIMORDIA STAGE

ELEMENT	ROOTS		LEAVES		STEMS	
	%	mg.	%	mg.	%	mg.
			Control Series			
Ca	1.47	2.94	4.14	49.68	3.24	8.10
K	5.52	11.04	5.78	69.36	10.24	25.60
Mg	0.69	1.38	0.77	9.24	0.87	2.18
N	3.28	6.56	6.74	80.88	2.64	6.60
P	0.85	1.70	1.07	12.84	0.78	1.95
			−Mg Series			
Ca	0.75	4.65	3.12	34.32	1.47	3.23
K	3.44	21.33	4.36	47.96	10.48	23.06
Mg	0.87	5.39	1.25	13.75	1.09	2.40
N	2.50	15.50	5.20	57.20	2.26	4.97
P	0.93	5.77	1.06	11.66	0.64	1.41
			−K Series			
Ca	1.32	4.36	3.93	40.48	2.62	5.24
K	4.70	15.51	5.80	59.74	10.54	21.08
Mg	0.76	2.51	0.74	7.62	0.82	1.64
N	2.46	8.12	5.15	53.05	1.95	3.90
P	1.04	3.43	1.06	10.92	0.66	1.32
			−Ca Series			
Ca	1.16	5.22	4.40	35.20	3.49	5.93
K	2.98	13.41	4.64	37.12	9.10	15.47
Mg	0.66	2.97	0.76	6.08	0.88	1.50
N	2.60	11.70	6.68	53.44	2.20	3.74
P	0.59	2.66	1.04	8.32	0.55	0.94
			−P Series			
Ca	1.57	11.78	3.77	62.21	2.76	9.11
K	2.00	15.00	2.20	36.30	5.68	18.74
Mg	0.62	4.65	0.74	12.21	0.92	3.04
N	3.20	24.00	4.55	75.08	1.95	6.44
P	1 09	8.18	1.01	16.67	0.78	2.57

stage (table IX). At the first sampling the plants were in a period of rapid vegetative growth while at the second sampling flower buds had become visible to the eye. The flowering (anthesis) samples were taken after several flowers at the lowest inflorescence had reached anthesis, early fruit enlargement samples when the ovaries of these flowers had begun to enlarge and the mid-fruiting samples when fruits of the first inflorescence were fully enlarged and had just turned red.

At each sampling, plants were divided into roots, stems and leaves including petioles. In the final harvest, fruits were also sampled separately.

Percentage dry weight of these portions was determined in a Brabender Moisture Tester at a temperature of 100° C. Leaf areas were determined at each sampling by tracing the leaf outlines on paper and computing areas with a planimeter. Plant material for chemical analysis was killed at a temperature of 100° C, dried rapidly at 80° C in a forced draft oven and ground to 40 mesh in a Wiley mill. Chemical analyses for magnesium, po-

TABLE VII

ELEMENTAL COMPOSITION OF VARIOUS PLANT PORTIONS AS PERCENTAGE OF DRY WEIGHT AND TOTAL QUANTITY IN MILLIGRAMS AT THE FLOWERING STAGE (ANTHESIS)

Element	Roots		Leaves		Stems	
	%	mg.	%	mg.	%	mg.
			Control Series			
Ca	1.69	74.36	3.43	459.62	1.65	59.40
K	2.12	93.28	3.28	439.52	3.76	135.36
Mg	0.50	22.00	0.53	71.02	0.45	16.20
N	3.56	156.64	5.73	767.82	3.55	127.80
P	0.98	43.12	0.63	84.42	0.59	21.24
			−Mg Series			
Ca	0.89	54.29	3.49	471.15	2.12	72.08
K	2.20	134.20	4.26	575.10	5.20	176.80
Mg	0.47	28.67	0.49	66.15	0.50	17.00
N	3.24	197.64	6.30	850.50	3.83	130.22
P	0.81	49.41	1.03	139.05	0.78	26.52
			−K Series			
Ca	0.97	83.42	3.87	634.68	2.21	101.66
K	1.42	122.12	2.98	488.72	5.01	230.46
Mg	0.47	40.42	0.44	72.16	0.41	18.86
N	1.88	161.68	4.19	687.16	2.36	108.56
P	0.55	47.30	0.96	157.44	0.64	29.44
			−Ca Series			
Ca	0.46	15.18	3.05	341.40	1.48	74.00
K	2.68	84.44	3.20	358.40	3.60	180.00
Mg	0.47	15.51	0.57	63.84	0.55	27.50
N	4.44	146.52	5.58	624.96	4.19	209.50
P	0.81	26.73	0.93	104.16	0.84	42.00
			−P Series			
Ca	1.19	86.87	4.37	638.02	2.65	87.45
K	1.60	91.98	2.42	353.32	2.88	95.04
Mg	0.66	48.18	0.59	86.14	0.59	19.47
N	5.08	370.84	6.60	96.36	4.55	150.15
P	0.78	56.94	0.91	132.86	0.69	22.77

tassium, calcium and phosphorus were made by modification of the methods of WOLF and ICHISAKA (**27**) and for nitrogen by modification of the method of KOCH and MCMEEKIN (**13**).

Data and discussion

NUTRIENT SOLUTION RESIDUES

Transplanted seedlings immediately began rapid growth. There soon were definite indications from the analyses of the jar residues (table IV)

that ions previously adsorbed on the pumice had been released to the nutrient solution (**11, 12**). This was particularly evident in the –K, –Ca and –P series in which large quantities of the adsorbed ions were found in the first solution residues. The uniformly healthy appearance of the plants confirmed this observation as none of them developed nutrient deficiencies during the first several weeks of growth. During the first six weeks it also

TABLE VIII

ELEMENTAL COMPOSITION OF VARIOUS PLANT PORTIONS AS PERCENTAGE OF DRY WEIGHT AND TOTAL QUANTITY IN GRAMS AT THE EARLY FRUIT ENLARGEMENT STAGE

ELEMENT	ROOTS		LEAVES		STEMS	
	%	gm.	%	gm.	%	gm.
			Control Series			
Ca	1.91	0.149	3.12	1.030	1.69	0.198
K	2.20	0.172	2.53	0.832	2.20	0.257
Mg	0.71	0.154	0.53	0.175	0.34	0.040
N	2.21	0.172	2.13	0.703	1.29	0.151
P	1.08	0.084	0.59	0.195	0.55	0.064
			–Mg Series			
Ca	1.06	0.144	3.30	1.109	1.47	0.162
K	1.62	0.220	3.28	1.102	2.92	0.321
Mg	0.36	0.049	0.53	0.178	0.42	0.046
N	1.86	0.253	2.25	0.756	1.78	0.196
P	0.56	0.076	0.78	0.262	0.59	0.065
			–K Series			
Ca	0.93	0.099	3.93	1.152	1.89	0.178
K	0.94	0.100	2.10	0.615	1.94	0.182
Mg	0.63	0.068	0.66	0.193	0.56	0.053
N	2.46	0.261	2.68	0.785	1.50	0.141
P	0.61	0.065	0.61	0.179	0.50	0.047
			–Ca Series			
Ca	0.35	0.026	2.40	0.538	0.57	0.051
K	3.52	0.264	3.36	0.753	2.20	0.196
Mg	0.49	0.037	0.76	0.170	0.55	0.049
N	3.08	0.231	3.25	0.728	2.48	0.221
P	1.08	0.081	0.85	0.190	0.72	0.064
			–P Series			
Ca	0.63	0.081	3.62	1.104	1.43	0.109
K	1.24	0.159	2.20	0.671	1.82	0.138
Mg	0.77	0.099	0.77	0.235	0.49	0.037
N	2.51	0.321	2.68	0.817	2.25	0.171
P	0.36	0.046	0.37	0.113	0.42	0.032

was noted that solution residues from all pumice series had a lower pH than that of the original solution. Stout working with hemp observed a similar pH shift in the jar residues (**23**). This response was in contrast to the controls in gravel which exhibited a rise in pH (fig. 1). Correlated with the rise in pH after the sixth week was extreme diminution in the amount of the various elements originally adsorbed on the pumice which were recovered in the nutrient solution residues (table IV).

A large fraction of the calcium found in the first week's residues of most of the pumice series was undoubtedly due to the soluble calcium derived from the pumice itself. This was indicated not only by leaching data, but also indirectly by the fact that at the end of the second week the concentration of calcium in these residues for most of the series had dropped by 50%. An additional fact pointing in this direction was that in some series, particularly –K, the quantity of calcium in the jar residues during the first four weeks was double that added in the original solution as shown by comparisons between tables III and IV. In comparing data on the composition of the nutrient solutions and that of plant tissue at the stem elongation phase (tables IV and V), it is interesting to note that high concentrations of potassium in the substrate are correlated with low calcium content in the tissues. This phenomenon of diminution in absorption of one element in the

TABLE IX

ELEMENTAL COMPOSITION OF VARIOUS PLANT PORTIONS AS PERCENTAGE OF DRY WEIGHT AND TOTAL COMPOSITION IN GRAMS AT THE MID-FRUITING STAGE

Element	Roots		Leaves		Stems		Fruit	
	%	gm.	%	gm.	%	gm.	%	gm.
				Control Series				
Ca	2.77	0.255	4.15	1.926	1.76	0.308	0.40	0.167
K	1.38	0.127	1.82	0.845	1.30	0.228	4.00	1.672
Mg	0.48	0.044	0.62	0.288	0.40	0.070	0.08	0.033
N	1.88	0.173	1.71	0.793	0.96	0.168	2.68	1.120
P	1.18	0.109	0.46	0.218	0.43	0.075	0.74	0.309
				–Mg Series				
Ca	2.36	0.236	3.82	1.627	2.03	0.371	0.43	0.186
K	0.90	0.090	2.12	0.903	1.14	0.208	4.18	1.810
Mg	0.07	0.007	0.42	0.179	0.26	0.048	0.10	0.043
N	1.65	0.165	1.88	0.801	0.83	0.152	1.85	0.801
P	0.78	0.078	0.59	0.251	0.47	0.086	0.71	0.307
				–K Series				
Ca	1.88	0.328	4.41	3.136	1.66	0.305	0.35	0.142
K	1.90	0.157	0.98	0.697	0.74	0.136	2.30	0.934
Mg	0.44	0.077	0.75	0.533	0.40	0.073	0.05	0.020
N	2.05	0.357	2.05	1.458	1.15	0.211	1.78	0.723
P	0.59	0.103	0.51	0.363	0.38	0.070	0.48	0.195
				–Ca Series				
Ca	0.71	0.105	1.77	0.660	0.60	0.100	0.38	0.078
K	1.34	0.199	2.18	0.813	0.88	0.147	3.12	0.644
Mg	0.23	0.034	0.86	0.321	0.52	0.087	0.11	0.023
N	2.50	0.371	3.01	1.123	2.01	0.335	1.88	0.388
P	0.62	0.092	0.91	0.339	0.72	0.120	0.67	0.138
				–P Series				
Ca	1.40	0.192	4.07	1.679	1.30	0.266	0.28	0.077
K	0.72	0.099	1.20	0.495	0.76	0.155	2.02	0.553
Mg	0.50	0.079	0.58	0.239	0.29	0.059	0.05	0.014
N	1.99	0.273	2.50	1.031	1.19	0.243	1.99	0.544
P	0.31	0.043	0.31	0.128	0.28	0.057	0.45	0.123

presence of an abundance of another (antagonism) has been observed by numerous workers (**5, 8, 9, 15**).

The amount of soluble calcium in the substrate is extremely variable, as shown by analyses for this ion in the –Ca series in which the original nutrient solution was lacking in calcium (table III). The total quantity of calcium present in the substrate at the end of the growing period was greater than the quantity determined as initially present (table II). Since analyses made before and after plant growth were determined for different samples of pumice, the variable leachability of calcium from the natural

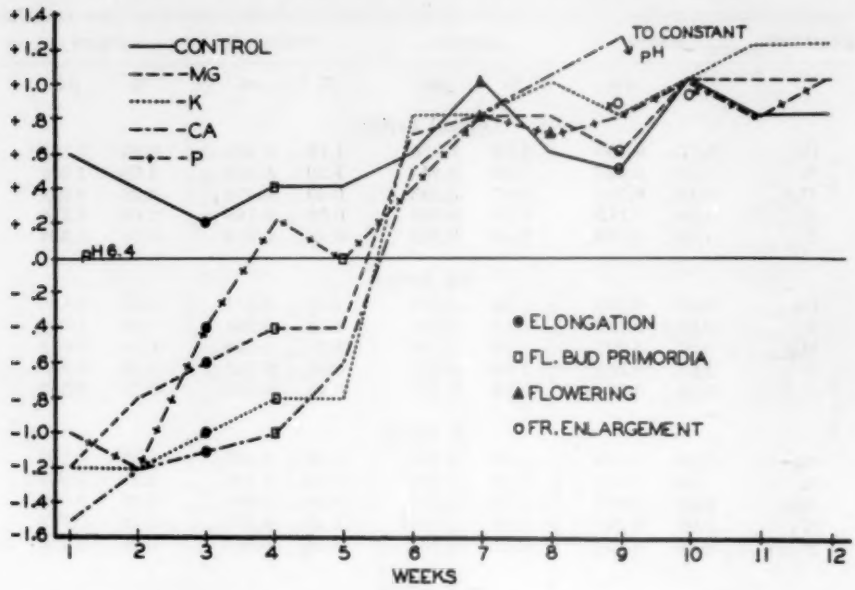

FIG. 1. Shifts in pH of the nutrient solutions. Determinations made with a LaMotte Roulette Comparator.

pumice became distinctly evident. After the seventh week, the quantity of originally adsorbed elements found in the nutrient solution was very limited, indicating almost complete cessation of release of these ions by the pumice to the nutrient solution.

During the flower bud initiation phase, larger quantities of nitrogen and phosphorus were found in the residues than were contained in the nutrient solution supply of the current week, suggesting some elimination of these elements from the plants (tables III and IV). Other investigators have also reported the apparent loss of ions by the plants to the soil solution during this developmental phase (**7, 10, 14, 19**).

GROSS MORPHOLOGY

STEMS.—Until the 12th week of plant growth, the stem height of the –Mg and –K plants exceeded the controls (table X). During the 12th week

TABLE X

MEAN STEM HEIGHT, NUMBER OF NODES AND LEAVES FOR EXPERIMENTAL SERIES AT WEEKLY INTERVALS

Weeks	Control series			–Mg series			–K series			–Ca series			–P series		
	Stem height in cm.	Number of nodes	Number of leaves	Stem height in cm.	Number of nodes	Number of leaves	Stem height in cm.	Number of nodes	Number of leaves	Stem height in cm.	Number of nodes	Number of leaves	Stem height in cm.	Number of nodes	Number of leaves
2	7.0	4	6	7.7	4	5	7.0	4	5	7.2	3	6	5.8	3	5
3	12.7	9	8	12.4	7	7	13.6	8	7	11.5	8	7	10.3	7	6
4	16.8	11	10	16.6	10	9	18.4	11	10	15.3	10	9	12.5	9	8
5	26.9	14	13	26.3	13	12	28.4	14	12	24.9	13	12	20.1	11	11
6	37.2	18	15	37.9	17	14	40.8	18	15	35.8	16	14	28.3	15	13
7	47.5	20	16	48.2	20	16	51.6	20	17	44.2	19	16	36.5	18	15
8	57.2	24	18	56.6	24	18	62.4	23	19	52.0	22	17	47.2	21	17
9	69.0	26	21	67.3	27	21	73.8	28	22	58.0	25	20	57.4	24	19
10	75.3	29	22	75.5	29	22	78.8	29	23	69.5	29	21	69.0	28	21
11	81.0	31	23	82.3	32	23	83.0	29	23	77.3	32	21	80.3	31	23
12	87.3	33	25	90.0	34	23	86.3	30	23	83.5	33	22	89.3	33	24

the rate of stem elongation of the –K series showed a considerable decline. Correlated with this reduction in rate of stem elongation was a diminished quantity of potassium in the stem (table IX). The stem elongation rate of the –Ca series of plants was slightly less than that of the controls throughout the growth period. In the –P series the stem elongation rate was below that of the controls in early developmental stages but finally surpassed the controls during the 12th week.

Control, –Mg, –K, –Ca and –P series of plants all maintained about the same rate of node initiation (table X). The mean internodal length in –K and –P series was slightly greater than the control at the 12th week, though not significantly so. The –Ca series exhibited a slightly shorter internodal length.

LEAVES.—Taking into account the number of leaves that abscised, the total number of leaves initiated in all series was about the same (table X). The leaf area of the control plants exceeded that of all plants of the pumice series at the first sampling (elongation), but by the flower bud primordial stage the leaf area of the plants of the –Mg series was in excess of the controls (fig. 2). At the final fruit phase the leaf area of the –Mg series far exceeded that of the control plants. Leaf areas of the –K and –P series were slightly less than that of controls.

FLOWERS.—There was no significant difference in the number of flowers in comparable inflorescences of the various series. Flower abscission in the –Mg and –Ca series, however, was 9% greater than in the control, while in the –K series of plants it was about 1% greater. The –P series had 1% less flower abscission than the control.

DEFICIENCY SYMPTOMS.—In the seventh week some of the plants of the –Ca series began to show such signs of typical calcium deficiency as retarded vegetative growth and yellowing of the leaves of the upper part of the plant (**1**). By the ninth week these symptoms had become very severe in some of the plants of the –Ca series but were not evident in others of the same series. This fact points to the irregular availability to plants of the calcium from pumice. Other investigators have noted a similar irregular release of calcium from synthetic ion-exchange resins employed in mineral nutrition experiments. On the basis of improved plant growth and composition, a reduction in the pH of the substrate evidently improved uptake of calcium. Thus plants were apparently taking up little or no calcium from the substrate at pH 7.0, as shown by deficiency symptoms and low calcium content of tissues. Upon reduction of hydrion concentration of the nutrient solution to pH 4.5 by addition of small amounts of acid, however, some of the plants previously manifesting deficiency symptoms showed indications of early recovery. New leaves were produced, but often they were abnormally thick and showed a tendency to curl downward at the margins. Some plants did not respond favorably to acidification of the nutrient solution. Analyses indicated that this pH shift caused greater leaching of calcium from pumice in some of the jars than in others. Reasons for this irregular

response were not clear. The appearance of blossom-end rot in the –Ca series during the tenth week may also be regarded as an indication of calcium deficiency (**16, 22, 26**).

ROOTS.—As a general rule roots of plants grown on pumice were larger and more uniformly distributed throughout the substrate. The roots of the control plants in gravel were comparatively short and more abundant at the upper levels of the substrate. The roots of the plants of the –Ca series developed several thick laterals and displayed extensive browning with some decay, typical symptoms of calcium deficiency (**1**).

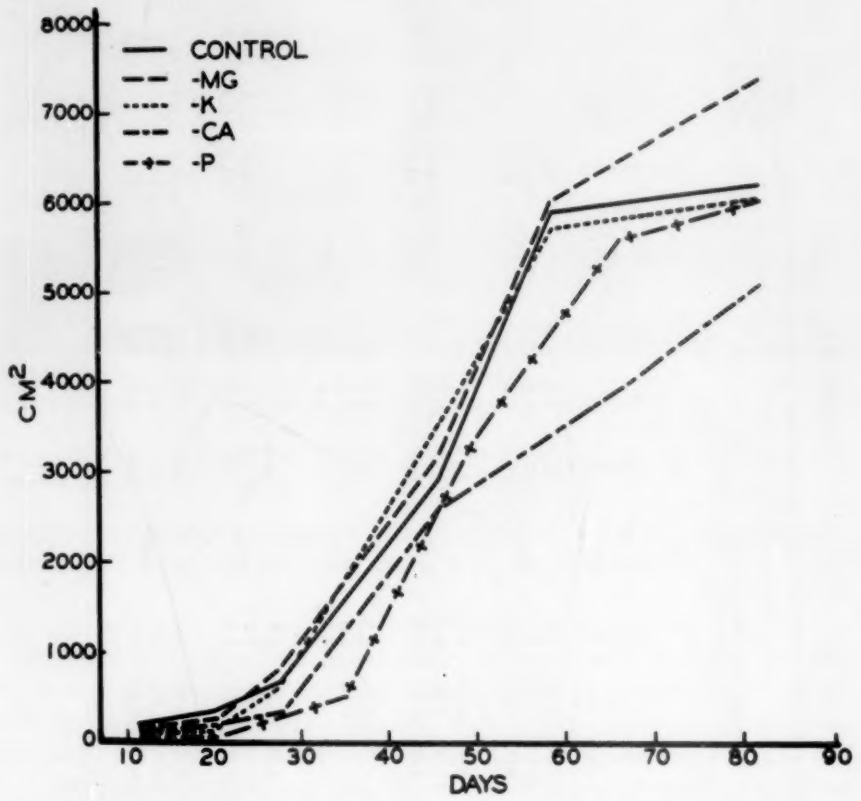

FIG. 2. Mean leaf areas of plants of the various experimental series.

FRESH AND DRY WEIGHTS.—Analyses based on fresh and dry weights of the various parts of plants indicated that the weights of roots of plants grown on pumice were consistently higher than for the controls in gravel (table XI). The weights of the shoots of all pumice series, with the exception of –Ca, were quite comparable to the controls. The shoots of the –Ca series of plants were consistently lower in weight. In all cases the top-root ratios of the plants grown on pumice were lower than the controls, because of the more extensive root systems of the pumice-grown plants (table XI).

TABLE XI

FRESH AND DRY WEIGHT IN GRAMS, PERCENTAGE DRY WEIGHT AND TOP-ROOT RATIO AT VARIOUS DEVELOPMENTAL STAGES OF THE EXPERIMENTAL SERIES

Series	Plant portion*	Stem elongation				Flower bud primordia				Flowering				Early fruit enlargement				Mid-fruiting			
		Fresh wt.	Dry wt.	% Dry wt.	T/R Ratio†	Fresh wt.	Dry wt.	% Dry wt.	T/R Ratio	Fresh wt.	Dry wt.	% Dry wt.	T/R Ratio	Fresh wt.	Dry wt.	% Dry wt.	T/R Ratio	Fresh wt.	Dry wt.	% Dry wt.	T/R Ratio
Cont.	R	0.83	0.05	5.9	8.20	1.60	0.20	7.6	7.25	41.9	4.4	10.6	3.86	89.2	7.8	8.8	5.73	101.1	9.2	9.1	11.49
	L	4.58	0.34	7.5		13.20	1.20	9.0		124.2	13.4	10.8		263.7	33.0	12.5		326.7	46.4	14.2	
	S	1.75	0.07	4.2		4.90	0.25	5.2		36.1	3.6	10.0		87.2	11.7	13.4		104.2	17.5	16.8	
	F	.....	.....	.....		.....	.....	.....		.....	.....	.....		.....	.....	.....		672.3	41.8	6.2	
–Mg	R	0.90	0.06	6.8	6.00	5.20	0.62	8.4	2.13	53.8	6.1	11.4	2.77	121.3	13.6	11.2	3.28	68.0	10.0	14.7	10.42
	L	3.68	0.29	7.3		11.90	1.10	9.0		128.7	13.5	10.5		258.0	33.6	11.8		373.6	42.6	11.4	
	S	1.65	0.07	4.2		4.60	0.22	5.0		42.0	3.4	8.2		81.8	11.0	13.4		109.9	18.3	16.6	
	F	.....	.....	.....		.....	.....	.....		.....	.....	.....		.....	.....	.....		721.8	43.3	6.0	
–K	R	1.36	0.10	7.4	4.10	4.70	0.33	6.7	3.73	80.0	8.6	10.7	2.44	96.1	10.6	11.0	3.65	138.3	17.4	12.6	7.47
	L	4.37	0.32	7.3		12.10	1.03	8.5		161.1	16.4	10.2		242.4	29.3	12.1		530.8	71.1	13.6	
	S	2.10	0.09	4.1		4.70	0.20	5.0		47.4	4.6	9.6		78.4	9.4	12.0		108.5	18.4	16.9	
	F	.....	.....	.....		.....	.....	.....		.....	.....	.....		.....	.....	.....		615.2	40.6	6.6	
–Ca	R	1.03	0.09	8.4	4.44	4.70	0.45	9.7	2.16	50.4	5.0	9.9	2.90	70.6	7.5	10.6	4.17	121.7	14.9	12.2	5.02
	L	4.00	0.32	8.1		10.10	0.80	7.9		114.6	11.2	9.8		188.0	22.4	11.9		305.8	37.3	12.2	
	S	1.70	0.08	4.4		3.60	0.17	4.6		36.4	3.3	9.2		61.3	8.9	14.5		108.1	16.7	15.4	
	F	.....	.....	.....		.....	.....	.....		.....	.....	.....		.....	.....	.....		282.8	20.6	7.3	
–P	R	0.73	0.07	9.4	3.86	8.50	0.75	8.8	2.64	68.3	7.3	10.6	2.45	101.2	12.8	12.6	2.98	118.3	13.7	11.6	6.49
	L	2.40	0.22	9.3		13.90	1.65	11.9		148.5	14.6	9.8		230.7	30.5	13.2		292.5	41.3	14.1	
	S	0.99	0.05	5.2		5.00	0.33	6.4		35.6	3.3	9.2		62.7	7.6	12.2		101.2	20.5	10.2	
	F	.....	.....	.....		.....	.....	.....		.....	.....	.....		.....	.....	.....		471.8	27.4	5.8	

* R—roots, L—leaves, S—stems and F—fruit.
† Based on dry weights.

Fruit produced by the first and second inflorescences of plants of the gravel control, –Mg, –K and –P series were all comparable in size. Fruits of the –Ca plants were much smaller than those of any other series (fig. 3). The mean fresh weight of fruits on plants in the gravel controls was greater than that for all pumice series except –Mg (table XI). The percentage dry

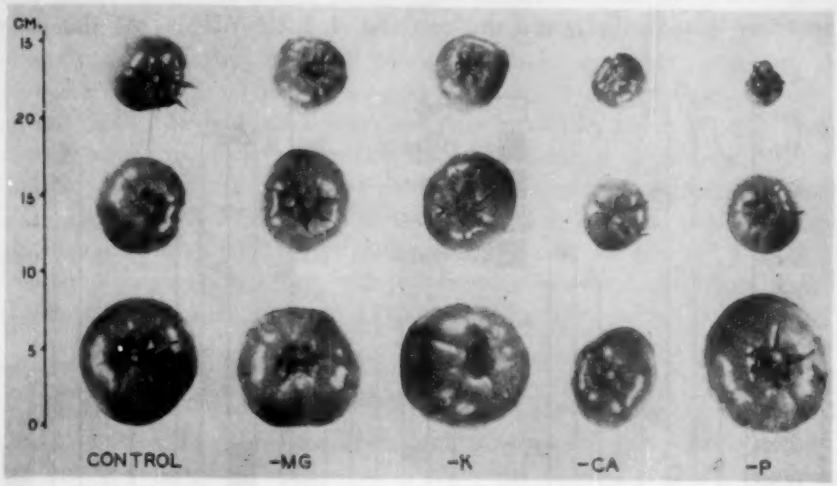

FIG. 3. Representative fruit at the mid-fruiting stage (82 days). First inflorescence bottom row, 28 day old fruit; second inflorescence center, 21 day old fruit in control, –Mg, –K and –Ca series, 14 day old fruit in –P series; third inflorescence top row, 14 day old fruit in control, –Mg and –K series, seven day old fruit in –Ca series, and four day old fruit in –P series.

weight of the fruits of the pumice series was about equal to that of the control except for the –Ca culture in which it was much higher.

TISSUE ANALYSIS

In the samples taken during the stage of rapid stem elongation it was found that the percentage of magnesium, potassium and phosphorus in the plants grown on pumice and which had been supplied these elements in the adsorbed form exceeded the corresponding values of the control series (table V). In these cases the total quantity of the elements present in the plant tissue was also greater (fig. 4). The percentage and total amount of calcium in the plants of the –Ca series was about the same as in the controls.

At the flower bud primordial stage the percentages and total quantities of magnesium and phosphorus were present in the plants of the adsorbed-ion series in equal or higher amounts than in the corresponding gravel controls (table VI). Although the percentage of potassium in the –K series and calcium in the –Ca series was higher in the leaves and stems, the total quantity thereof was less (fig. 4).

By the time the plants had reached the flowering stage, the only plants of the adsorbed-ion series which had a percentage of elements equal to or

greater than that found in control plants were the –Mg series, the stems of –K series, and the stems and leaves of the –P series (table VII). The total quantity of ions present when compared to control were higher in the –Mg, –K and –P series (fig. 4). In the fruit enlargement phase magnesium in the –Mg series was the only element supplied in an adsorbed form which occurred in the plants in equivalent or higher percentages and higher total quantities than in the gravel controls (fig. 4, table VIII). At this stage

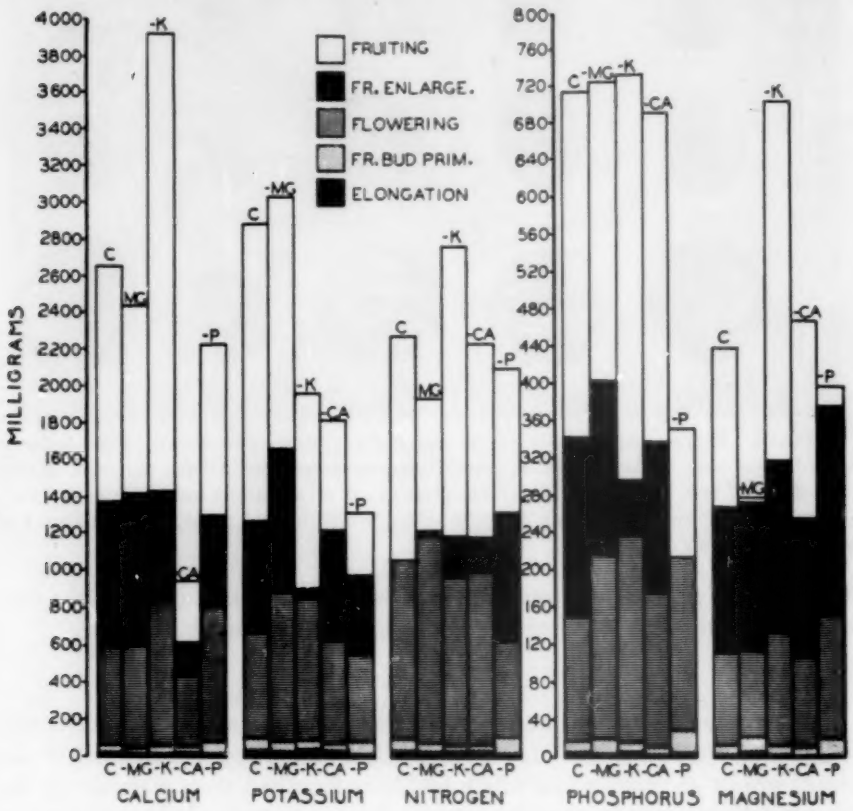

FIG. 4. Mineral increment of the tomato plants at various stages of development. Series grouped by elements: C, control; –Mg designates that all magnesium was originally supplied in adsorbed form on pumice in this series; same for –K, –Ca and –P respectively. Data based on tables V to IX.

the percentage of calcium present in the –Ca plants was about one-half that found in the controls and the total quantity was only about one-third. During the stage of rapid fruit enlargement, visible signs of extreme calcium deficiency were correlated with the low calcium content of tissue of the –Ca series.

In all cases in which an element had been supplied in an adsorbed form, it was found in a smaller quantity in all tissues during the fruiting stage

than in the corresponding control series (table IX), indicating that the total intake of the adsorbed element was less than from a nutrient solution containing all essential elements in the soluble form (**20**).

Certain striking transitions were observed in the composition of plants in the course of their development within a particular series. At the elongation phase in the –K series, there was a relatively high potassium concentration in the plant with a corresponding low calcium content (**4, 5, 6, 8, 15**), a condition reflecting the high concentration of available potassium ions (tables IV and V). This calcium deficiency (fig. 4) was also associated with an increased intake of magnesium and phosporus (**24, 25**). When plants in the –K series reached the fruiting stage, however, a potassium deficiency had developed and the intake of calcium had increased appreciably (tables VIII and IX) (**2, 17, 25**). This late increase in calcium intake was associated with a distinct gain in nitrogen content of the plants (**3**). In the –Ca series it was noted that with low intake of calcium there was a higher absorption rate of magnesium (**17**), but not of potassium.

Conclusions

On the basis of gross habit of the plants in the various series, it was found that growth in the –Mg, –K and –P pumice series was comparable to that in the gravel controls. The metabolic efficiency of tomato plants dependent upon the treated pumice for their supply of a given element was also comparable to that of the controls in gravel because the dry weight was essentially the same in both at maturity. Evidently sufficient quantities of magnesium, potassium and phosphorus were adsorbed on the pumice to satisfy the plant requirement for growth to maturity. On the other hand, the quantity of available calcium was inadequate for normal growth. In spite of the fact that plant growth was often comparable irrespective of whether an ion was originally supplied in an adsorbed or freely soluble form, the total quantity of an ion in the plant was less when supplied as a pumice-adsorption complex (**20**).

During the period that ions which had originally been adsorbed on the pumice were being rendered soluble, the plants were evidently wholly dependent upon this source of a given ion. After this time, however, due to weekly drainage and replacement of solutions, very small quantities or sometimes none of the originally adsorbed ions appeared in the nutrient solution. The plants nevertheless continued to absorb these elements in substantial amounts (fig. 5). To judge from increments of these ions in the plant tissues certain amounts of them were absorbed directly from the pumice-adsorption complex. An important factor in making adsorbed elements available to plants grown in pumice might well have been their more luxuriant root systems which were in extremely intimate contact with and ramified throughout the pumice to such a marked degree as to suggest a strong tropic stimulus. One consistent difference between the gravel and pumice-grown plants was a larger and more extensive root system in the

cultures containing pumice shown by the uniformly smaller top-root ratio for the plants of all pumice series.

In contrast with previous investigations (**18, 23**) in which complete nutrient solutions were added to a pumice substrate, there was no indication in the present experiment that a pumice substrate as treated herein greatly accelerated plant development or materially improved plant growth as compared with ordinary gravel or soil cultures.

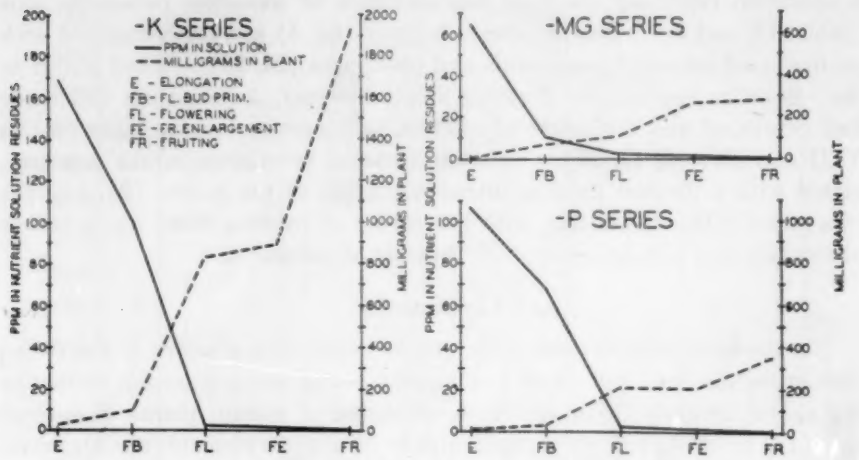

FIG. 5. Amounts of ions originally adsorbed on pumice which occur in nutrient solution residues and in entire tomato plants at various stages of development in the pumice series. Left, –K series shows potassium content of solution residues and of entire plants. Right, comparable data for magnesium and phosphorus in the –Mg and –P series.

Summary

1. This investigation employing the Pan America variety of tomato was undertaken to determine the availability for plant growth of ions of magnesium, potassium, calcium and phosphorus which had been adsorbed on a pumice substrate. Each of these ions was adsorbed on a separate lot of pumice and all the other essential elements were supplied in a nutrient solution. Four single ion pumice-adsorption complexes were employed and the series designated as –Mg, –K, –Ca and –P respectively. For comparison a control series with an inert quartz gravel substrate was also employed, to which all of the essential elements were supplied in nutrient solution.

2. Loss of appreciable amounts of previously absorbed nitrogen and phosphorus from tomato plants to the substrate was noted in all series at the flower initiation phase.

3. In the –K series, tissue content of calcium was low when the available potassium supply in the nutrient solution was high.

4. Compared with gravel, the pumice substrate as employed in this investigation did not enhance yield or accelerate maturation of tomato plants.

5. Plants in the –Ca series paralleled control plants grown in gravel in leaf and node number but plants of the –Ca series had a smaller total leaf area and were shorter than controls in stem height. Plants of the –Ca series exhibited symptoms of calcium deficiency.

6. Plants dependent upon magnesium, potassium and phosphorus adsorbed on pumice were comparable in stem height, total leaf area, number of leaves and number of nodes to those grown in gravel containing a complete nutrient solution. The total weight of fruit by plants in the –Mg and –K series produced in a 12-week growing period was comparable to gravel controls but slightly less than controls in the –P series.

7. Root systems of the plants grown in pumice substrates were much larger than those in the gravel controls. Roots of the pumice-grown plants ramified throughout the individual lumps of pumice and were in intimate contact with the pumice substrate as if responding to a strong tropic stimulus.

8. The total quantity of an element absorbed by the plants from a pumice-adsorption complex, treated as described, was less than when the ion was supplied in nutrient solution but plant growth was essentially comparable.

9. Pumice treated as described supplied sufficient amounts of available magnesium, potassium and phosphorus for normal growth but not enough of calcium. Calcium evidently is not held firmly by pumice, as indicated by the early leaching thereof into the nutrient solution. Pumice compares favorably with other artificial substrate used in nutritional studies. It is cheaper than most solid substrates, a fact which may permit use of pumice in large scale experimental and commercial operations.

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SEASONAL FLUCTUATIONS OF ROOT RESERVES IN RED CLOVER, *TRIFOLIUM PRATENSE* L.[1]

DALE SMITH

(WITH ONE FIGURE)

Received April 11, 1950

Medium red clover behaves primarily as a biennial under the two-cutting system of management usually practiced in the principal area of its culture in the United States. In some areas, where both soil and climatic conditions are highly favorable, stands of medium red clover are known to persist for two or more years following the year of establishment. This occurs most frequently when virgin soil is brought into cultivation. Mammoth red clover, on the other hand, is a one-cut clover and the perennial habit of growth is more pronounced than with the medium red clover. At best, however, mammoth red clover behaves as a very short-lived perennial.

The periodic accumulation and diminution of reserve carbohydrates in the roots and crowns of leguminous plants has long been recognized as a means of indicating seasonal development, as well as the influence of management on both survival and productivity. Extensive studies have been made by GRABER *et al.* (**1**), SMITH and GRABER (**7**) and others on such legumes as alfalfa and sweet clover as to the effect of management on food storage and the effect of different levels of food storage on the growth of both roots and tops. Such studies with medium and mammoth red clover are less numerous and little information is available dealing with food reserves in relation to their biennial or perennial habit of growth. GREATHOUSE and STUART (**2, 3, 4**) have studied the overwintering physical and chemical composition of medium red clover and have found a high level of food storage to be one of the factors associated with winterhardiness. VIRTANEN and NURMIA (**9**), working in Finland, have shown that carbohydrate reserves are used and then restored in the roots following the cutting of red clover in the second season and that the roots were well stored with carbohydrates by the end of the second season under the treatments used. The reserve carbohydrate trends presented by Virtanen and Nurmia would suggest that red clover reacts physiologically as a perennial.

The investigations reported herewith were made to obtain further evidence relating to the physiological behavior of medium and mammoth red clover as perennials or biennials. Such evidence was obtained by determining the seasonal fluctuations of food reserves in the roots. Periodic samplings were made during the growing seasons and during the dormancy of winter months. The food reserves were then measured in terms of the percentages of total available carbohydrates and total nitrogen.

[1] Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

Materials and methods

Wisconsin strains of medium and mammoth red clover were seeded at Madison, Wisconsin, on Miami silt loam soil of only moderate fertility. Plots of each clover were established in randomized complete blocks with four replications. The clovers were seeded broadcast on May 7, 1947, at the rate of 18 pounds per acre. An oat companion crop was sown at 1 bushel per acre prior to the seeding of the clovers. On June 27, the companion crop was removed just above the clover top growth. The clovers were then allowed to grow undisturbed during the remainder of the first season.

Various management treatments were applied during the second season of growth. With the medium red clover, one series of plots received no cutting. Three separate series of plots were cut at full bloom on July 1. The second hay crop was then cut at full bloom on August 18 in one series, at the seed stage on September 2 in the second series, and the third series received no second cutting. With the mammoth red clover, one series of plots received no cutting and one series was cut once at full bloom on July 16.

Changes in the stands under the treatments were determined by plant counts. Eight random 2 square feet quadrats were taken in each plot.

At approximately two-week intervals during the first and second seasons of growth, 50 representative plants were dug from each replicate of the different treatments. The roots were washed, cut to a uniform length of eight inches and the tops were cut off at the junction of root and crown. The roots were partially dried under forced ventilation at 100° C for one hour and drying was completed at 80° C. The oven-dry roots from all replicates under the same treatment were bulked together and finely ground in a Christy-Morris Mill.

Carbohydrate analyses were made on each root sample in duplicate to determine the total available carbohydrates. The Weinmann method (**8**) of total available carbohydrate extraction was used and the modifications outlined by LINDAHL *et al.* (**6**) were followed. The reducing power was determined by the Shaffer-Somogyi method as outlined by HEINZE and MURNEEK (**5**) and the total available carbohydrates were calculated as glucose. Total nitrogen analyses were made in duplicate using the Kjeldahl method. The percentages of total available carbohydrates and total nitrogen in the roots were calculated on a dry weight basis.

Percentages of total available carbohydrates

The percentages of total available carbohydrates (TAC) in the roots of medium and mammoth red clover during the first and second seasons of growth for the various management treatments are tabulated in table I. The trends of TAC for the management treatments most commonly used in the culture of medium and mammoth red clover are shown in figure 1.

The analyses indicate that the trends of reserve carbohydrates in the roots of both medium and mammoth red clover were essentially alike where similar treatments were applied. However, the percentages at comparable sampling dates were generally lower for the mammoth red clover.

The percentages of TAC in the roots of both medium and mammoth red clover increased during the seedling year to a maximum in mid-October and for the remainder of the first season they declined slowly. Also,

TABLE I

THE PERCENTAGES OF TOTAL AVAILABLE CARBOHYDRATES IN THE ROOTS OF MEDIUM AND MAMMOTH RED CLOVER DURING THE FIRST AND SECOND SEASONS OF GROWTH AS INFLUENCED BY VARIOUS MANAGEMENT TREATMENTS

Dates of sampling	Medium red clover				Mammoth red clover	
	Not cut	Cut July 1 1948	Cut July 1 and August 18 1948	Cut July 1 and September 2 1948	Not cut	Cut July 16 1948
			1947			
July 15	20.0	.....	.....	.....	14.2	.....
August 1	24.5	.....	.....	.....	19.8	.....
August 15	28.5	.....	.....	.....	21.7	.....
September 2	28.0	.....	.....	.....	21.7	.....
September 15	25.3	.....	.....	.....	20.7	.....
October 1	30.2	.....	.....	.....	25.5	.....
October 15	36.2	.....	.....	.....	32.6	.....
October 30	34.9	.....	.....	.....	32.4	.....
November 15	31.9	.....	.....	.....	29.7	.....
			1948			
April 1	13.6	.....	.....	.....	9.6	.....
April 19	9.8	.....	.....	.....	8.4	.....
May 3	12.5	.....	.....	.....	7.8	.....
June 1	17.8	.....	.....	.....	9.7	.....
June 17	23.5	.....	.....	.....	16.4	.....
July 1	28.3	28.3	28.3	28.3	20.3	.....
July 16	31.5	14.3	14.3	14.3	22.1	22.1
August 3	21.2	16.6	16.6	16.6	16.9	6.4
August 18	21.3	21.2	21.2	21.2	14.8	11.4
September 2	23.2	25.8	18.6	25.8	15.2	18.1
September 20	.....	.....	17.6	18.1	.....	.....
October 1	22.1	25.1	18.8	23.1	15.5	23.0
November 15	30.0	28.5	31.0	27.7	25.5	30.0

there was a slight decline in the percentages during late August and early September which corresponded to a period of very limited rainfall. Apparently, growth and accumulation of carbohydrates were retarded during this dry summer period and the percentages of TAC in the roots were decreased through respiratory losses.

A very marked reduction occurred in the percentages of TAC in the roots during the winter months, as measured between November 15 and April 1. At this early spring date no new growth was in evidence. Yet, during this period, the percentage of TAC in the roots of medium red clover declined from 31.9 to 13.6% and from 29.7 to 9.6% in the roots of

mammoth red clover. These available carbohydrates were reduced to a much lower level during winter dormancy than has been reported by GRABER *et al.* (1) for the long-lived perennial alfalfa. This would seem to indicate that medium and mammoth red clover maintain a high rate of metabolic activity during winter dormancy. As a result, red clover would appear to be readily predisposed to winter injury and to diseases, particularly during the late winter and early spring with the advent of low carbohydrate reserves. An average of 60.0% of the clover plants failed to survive the first winter of this study.

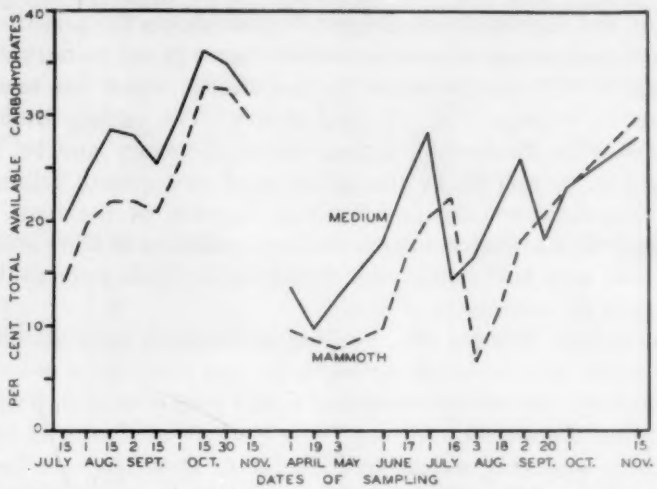
PER CENT TOTAL AVAILABLE CARBOHYDRATES
40
30
20
10
0
MEDIUM
MAMMOTH
15 1 15 2 15 1 15 30 15 1 19 3 1 17 1 16 3 18 2 20 1 15
JULY AUG. SEPT. OCT. NOV. APRIL MAY JUNE JULY AUG. SEPT. OCT. NOV.
DATES OF SAMPLING

FIG. 1. The trend of total available carbohydrates in the roots of medium red clover cut in the second year at full bloom on July 1 and for seed on September 2, and mammoth red clover cut in the second year only at full bloom on July 16.

The analyses made after April 1 in the second year show that the succulent spring growth of red clover reduced the percentages of TAC in the roots to still lower levels and these low levels were reached in mid-April for medium red clover and about two weeks later for mammoth. Subsequently, as sufficient photosynthetic area was produced, the percentages of TAC increased during May and June reaching a high level of storage again in mid-July. By mid-July, the medium red clover was at the seed stage and the mammoth was at full bloom. GREATHOUSE and STUART (**3, 4**), sampling medium red clover from the field at intervals of approximately two months from September 10 at the seedling year to July 15 of the second year, have also shown these same general carbohydrate trends in terms of the readily available carbohydrates (sugars, starch and dextrins).

With the red clover plants left uncut for the entire second season, the percentages of TAC in the roots declined after mid-July with the deterioration of the first crop top growth and with the production of new basal shoots. However, these carbohydrates were later restored and continued

to increase until late fall. Likewise, all cutting treatments made during the second season brought about marked fluctuations in the percentages of TAC in the clover roots. Following each cutting, the percentages of TAC in the roots declined as they were utilized primarily for the development of new succulent top growth. Subsequently, the percentages increased as the amount of recovery top growth became more abundant. Also, under all cutting treatments, the roots of both medium and mammoth red clover entered the second winter with a high level of carbohydrate storage. In this respect, medium and mammoth red clover behave quite differently than the biennial legume, sweet clover (**7**).

Medium and mammoth red clover have both shown the periodic accumulations and diminutions of reserve carbohydrates in the roots that are usually associated with the perennial legume alfalfa, which has been studied extensively by GRABER *et al.* (**1**) and others. The carbohydrate reserves in the roots were diminished during winter dormancy and by the early spring growth, as well as, by the initiation of new growth following each cutting. Furthermore, the carbohydrate reserves in the roots were restored whenever the photosynthetic area was sufficient to allow such storage and the roots were well stored with carbohydrate foods prior to the second winter under all treatments.

VIRTANEN and NURMIA (**9**), working in Finland, have also shown the diminution and restoration of carbohydrate root reserves in red clover following cutting in the second season and a high level of carbohydrate storage upon entering the second winter. These workers used cutting treatments during the second year which were similar to those made in the current study, namely, one plot was left uncut, one plot was cut once on June 26, and three plots were cut on June 26 with second cuttings being made on August 26, September 7 and October 4. However, Virtanen and Nurmiä did not study the trend of carbohydrates in the seedling year or over winter.

Percentages of total nitrogen

As with the carbohydrates, the seasonal trends in the percentages of total nitrogen in the roots of medium and mammoth red clover were relatively the same when compared under similar management treatments (table II). However, the fluctuations in the percentages of total nitrogen were very small as compared to the changes which occurred in the percentages of total available carbohydrates. The data also show that the percentages of total nitrogen in the roots declined or increased under the various treatments in a manner similar to the carbohydrates. However, the dates of the maximum or minimum content of total nitrogen in the roots occurred somewhat later than the corresponding dates for the carbohydrates. There was one major exception. During the winter, the percentages of total nitrogen in the roots increased while there was a marked decline in the percentages of total available carbohydrates. This is in agreement with the chemical analyses made on medium red clover by

GREATHOUSE and STUART (**2, 3, 4**). The respirational losses of carbohydrates over winter probably account, in a large part, for the increase in the percentages of total nitrogen.

Changes in stand

Excellent stands of medium and mammoth red clover were obtained in the seedling year, as measured on November 14, 1947 (table III). Plant

TABLE II

THE PERCENTAGES OF TOTAL NITROGEN IN THE ROOTS OF MEDIUM AND MAMMOTH RED CLOVER DURING THE FIRST AND SECOND SEASONS OF GROWTH AS INFLUENCED BY VARIOUS MANAGEMENT TREATMENTS

DATES OF SAMPLING	MEDIUM RED CLOVER				MAMMOTH RED CLOVER	
	NOT CUT	CUT JULY 1 1948	CUT JULY 1 AND AUGUST 18 1948	CUT JULY 1 AND SEPTEMBER 2 1948	NOT CUT	CUT JULY 16 1948
			1947			
July 15	2.38	.....	.....	.....	2.38	.....
August 1	.....	.....	.....	.....	.....	.....
August 15	2.36	.....	.....	.....	2.39	.....
September 2	2.24	.....	.....	.....	2.29	.....
September 15	2.11	.....	.....	.....	2.24	.....
October 1	2.14	.....	.....	.....	2.05	.....
October 15	2.06	.....	.....	.....	2.21	.....
October 30	2.51	.....	.....	.....	2.46	.....
November 15	2.58	.....	.....	.....	2.68	.....
			1948			
April 1	3.04	.....	.....	.....	3.47	.....
April 19	2.57	.....	.....	.....	2.51	.....
May 3	1.99	.....	.....	.....	1.91	.....
June 1	1.99	.....	.....	.....	1.93	.....
June 17	2.10	.....	.....	.....	2.13	.....
July 1	2.29	2.29	2.29	2.29	2.28	.....
July 16	2.41	2.38	2.38	2.38	2.39	2.39
August 3	2.43	2.05	2.05	2.05	2.45	2.28
August 18	2.49	2.20	2.20	2.20	2.53	2.21
September 2	2.58	2.20	2.44	2.20	2.41	2.31
September 20	.....	.....	2.22	2.24	.....	.....
October 1	2.12	2.16	2.01	1.97	2.16	2.15
November 15	2.30	2.17	2.17	2.18	2.18	2.23

counts made the following spring on May 7, 1948, before any cutting treatments were applied, show that a large loss of plants occurred during the first winter. This overwinter loss was about the same for both clovers and averaged 60.0% for all plots.

Plant counts made on October 29, 1948, after all second year treatments had been completed, indicate that a general decline occurred in the stands during the growing season. Counts made on April 20, 1949, the third year, show that a considerable number of clover plants survived the second winter. In this respect, some of the clover plants were capable of manifesting a true perennial habit of growth.

The only significant difference between treatments in plant population was where the clovers were not cut. The stands of both medium and mammoth red clover under this treatment were significantly lower by the end of the second season. This extra loss of plants appears to have resulted through excessive transpiration from the uncut top growth during the dry summer months. These lower stands in the uncut plots continued to be apparent in the spring of the third year.

TABLE III

CHANGES IN THE PLANT POPULATIONS OF MEDIUM AND MAMMOTH RED CLOVER AS INDICATED BY THE AVERAGE NUMBER OF CLOVER PLANTS PER TWO SQUARE FEET

CLOVER AND TREATMENTS	1947 NOVEMBER 14	1948 MAY 7	1948 AUGUST 20	1948 OCTOBER 29	1949 APRIL 20
Medium red clover					
Not cut	33.3	11.2	7.8	5.9	3.7
Cut July 1, 1948	28.6	14.1	9.3	8.9	5.0
Cut July 1 and August 18, 1948	34.2	15.2	10.5	9.2	6.6
Cut July 1 and September 2, 1948	29.5	11.2	8.4	8.7	4.7
Mammoth red clover					
Not cut	29.3	10.7	5.3	3.3	1.8
Cut July 16, 1948	27.2	10.4	8.1	7.2	4.6
L.S.D. 5% level	n.s.	n.s.	2.9	2.0	1.2

Summary

The seasonal fluctuations of root reserves in medium and mammoth red clover were studied at Madison, Wisconsin, during 1947 and 1948, to obtain evidence relating to the physiological behavior of these legumes as perennials or biennials. The percentages of total available carbohydrates and total nitrogen were determined on root samples collected from the field at approximately two-week intervals during the first and second seasons of growth.

Growth during the first season was left undisturbed, but various management treatments were applied in the second year. With medium red clover, one set of plots was left uncut, one set was cut once at full bloom on July 1, one set was cut at full bloom on July 1 and August 18, and a final set of plots was cut at full bloom on July 1 and at the seed stage on September 2. With mammoth red clover, one set of plots was left uncut and another set was cut once at full bloom on July 16.

The seasonal trends of food reserves in the roots of medium and mammoth red clover were essentially alike where similar treatments were applied. However, the percentages of total available carbohydrates at comparable sampling dates were generally lower for the mammoth red clover.

The seasonal fluctuations of root reserves in both medium and mammoth red clover under all treatments were generally typical of the re-

sponses usually associated with the perennial legume, alfalfa. The carbohydrate root reserves were diminished during winter dormancy and both carbohydrate and nitrogen root reserves were reduced by the early spring growth, as well as by the initiation of new growth following each cutting. Restoration of the food reserves in the roots occurred whenever the photosynthetic area was sufficient to allow such storage. Furthermore, the roots were well stored with reserve foods prior to the second winter under all of the treatments used. In this respect, medium and mammoth red clover behave differently than biennial sweet clover. Also indicative of the perennial behavior of medium and mammoth red clover is that a considerable number of plants of both clovers survived the second winter and initiated growth in the following spring.

Medium and mammoth red clover, however, differed from the known behavior of the long-lived perennial alfalfa in that the total available carbohydrates were reduced to a much lower level during winter dormancy. Perhaps this is one factor contributing to the short life of both medium and mammoth red clover such that they behave, at best, as short-lived perennials.

The writer expresses appreciation to Dr. J. H. Torrie and Dr. L. F. Graber, Professors of Agronomy, and Dr. E. W. Hanson, Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, for their helpful suggestions and to R. J. Bula for assistance in the carbohydrate analyses.

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IN VITRO TESTS OF ABSCISSION AGENTS[1]

GEORGE A. LIVINGSTON

(WITH EIGHT FIGURES)

Received March 20, 1950

Premature fruit fall and leaf fall have for centuries been a source of loss to growers, but in the last ten years methods have been developed which permit the control of these phenomena under many circumstances. It is now possible either to accelerate or to retard the rate of abscission of leaves and fruits by the application of various chemicals.

Detailed analyses of anatomy in relation to abscission have been made since the middle of the eighteenth century but very few investigations of the physiology of abscission have been attempted. In 1936 LA RUE (**4**) demonstrated that the abscission of debladed petioles could be delayed by application of growth substances. Since then a number of experiments have been reported on the regulation of abscission (**1, 2, 3, 4, 5, 7, 10, 11, 12**) but they include relatively little research on the fundamental physiology of abscission. It was the objective of this investigation to study the physiology of abscission with experiments *in vitro*.

Materials and methods

The experiments reported below employed the fully mature leaves of *Citrus sinensis* var. Valencia orange. The leaves of this variety are unifoliolate with two active abscission zones: (1) the laminar abscission zone, between the lamina (blade) and the petiole, and (2) the nodal abscission zone, between the petiole and stem. The laminar abscission zone is characterized externally by an encircling groove.

In all but one of the experiments the laminar abscission zone was excised and was used in this investigation. This zone was contained in a portion of tissue consisting of 6 mm. of the petiole and 10 mm. of the mid rib. The remaining blade tissue and the petiolar wings, if present, were removed. This excised portion of the leaf is subsequently referred to as the "explant." Such explants were mounted on agar in Petri dishes, the relation of the explant to the leaf and the method of mounting being shown in figure 1. The details of this method have already been reported (**1**).

Chemical agents were applied to the explants in either of two ways: (1) by dissolving the agent in the agar upon which the explants rested; or (2) by immersing the explants for known times in solutions of the chemical and then placing them on the agar.

Under field conditions the movement of leaves by air currents and other

[1] This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W-18-035-CM-208 with the University of California.

agents places significant stress upon the abscission layer. Since this stress was normally lacking from the *in vitro* experiments of this study it was considered advisable to replace the stress, at least in part. This was done by raising each unabscissed explant at the close of the experimental period, with the tips of a pair of forceps under the petiolar portion. On the graphs the data shown to the left of the broken vertical line are the daily percentages of unaided abscission while the data to the right of the line include that obtained after the application of the stress by lifting on the final day of

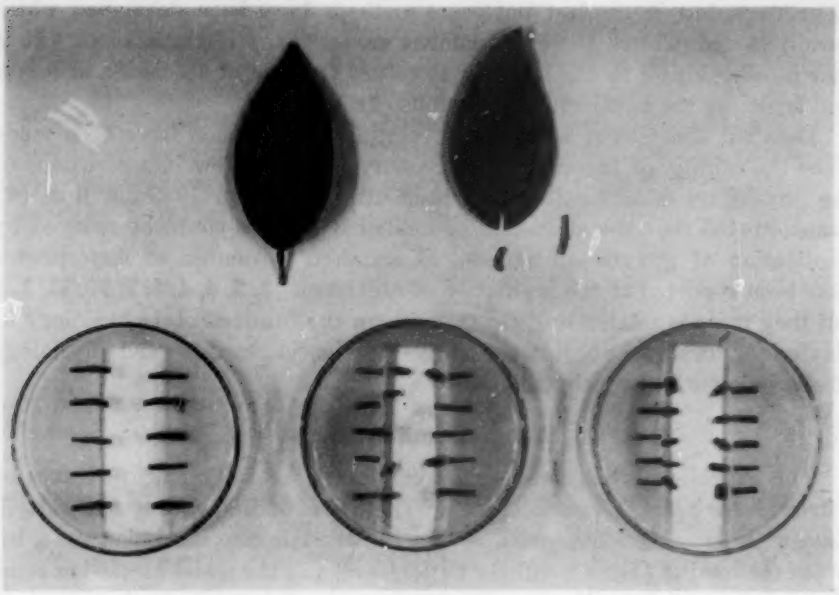

FIG. 1. Photograph showing a normal leaf, a leaf with an explant removed and three agar dishes with explants. The dishes show initial, intermediate and final stages in abscission.

the experiment. The data represented in the graphs are the percentages of abscission obtained from 20 explants per treatment.

Experiments and results

EFFECT OF ETHYLENE

It is common knowledge that ethylene can increase the rate of abscission of leaves from branches. To test the effects of ethylene on explants, Petri dishes with explants were sealed under bell jars of approximately six liters capacity. The atmospheres in the bell jars were modified in the following ways: A. Control (ordinary air). B. One ml. of water saturated with ethylene introduced into the bell jar at the start of the experiment. C. 10 ml. of water saturated with ethylene introduced at the start of the experiment. D. 10 ml. of water saturated with ethylene introduced 48 hours after the start of the experiment. (The ethylene solution employed was

saturated at 25° C.) Figure 2 presents the results. They show that ethylene increased the rate of abscission in excised tissues.

Effect of toxic substances

Pfeiffer (8) reported that a 1% solution of chloral hydrate promoted abscission. Defoliants are available which if applied under the proper conditions will bring about abscission. Chloral hydrate and cyanamid (Experimental Defoliant X-5 of the American Cyanamid Company) were applied separately to explants in order to test their effect on explant abscission. One per cent. chloral hydrate was dissolved in the agar which supported the explants. The cyanamid was dissolved in distilled water in concentrations

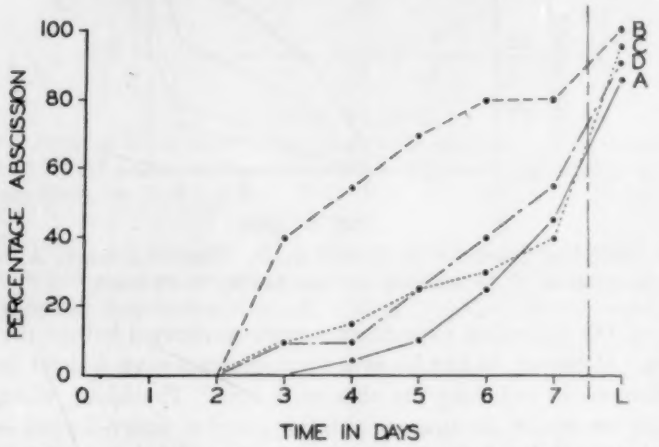

Fig. 2. Effect of ethylene. The explants were placed in bell jars (six liter capacity) and the atmosphere modified in the following ways: A. Control (ordinary air). B. One ml. of water saturated with ethylene added at the start of the experiment. C. 10 ml. of water saturated with ethylene introduced at the start of the experiment. D. 10 ml. of water saturated with ethylene added forty-eight hours after the start of the experiment.

varying from .0001 to 3.% and the explants were dipped momentarily in these solutions. The chloral hydrate and all concentrations of cyanamid inhibited abscission. The rate of inhibition of the various concentrations of cyanamid was directly proportional to the concentration of cyanamid.

Effect of immersion

In the preliminary experiments explants were placed in aqueous solutions. Under these conditions no abscission occured, even after many weeks. After adopting agar as a supporting medium it became of interest to learn more of the effect of immersion on abscission. An experiment was conducted in which explants were immersed in distilled water for the following periods: A. None (control). B. One minute. C. 30 minutes. D. Four hours. E. 24 hours. The explants were then mounted on agar and examined daily. The results are presented in figure 3. They show that immersion inhibits abscission and that the degree of inhibition is proportional

to the duration of the immersion period. These results must be borne in mind where explants have been treated by immersion in aqueous solutions. The immersion tests were repeated a second time in order to determine the reproducibility of results. There was a slight variation between the two tests as could be expected but on the whole the results were comparable.

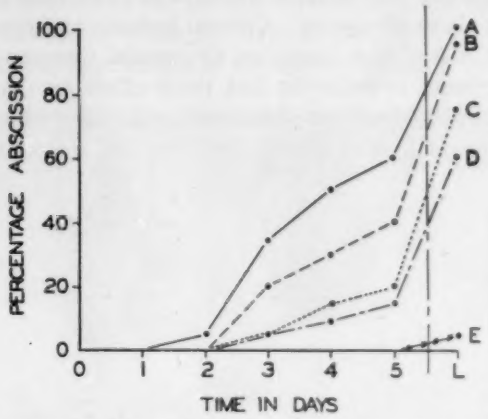

FIG. 3. Effect of immersion in distilled water. Immersion times: A. None (control). B. One minute. C. 30 minutes. D. four hours. E. 24 hours.

Many of the following experiments were conducted before the tests on immersion. However, as can be seen from figure 3 even a short immersion time is effective in reducing the abscission rate. Therefore, whenever the tests entail immersion in aqueous solutions and a water-dipped control is not included Line B of figure 3 should be consulted to determine what fraction of the effect is due to the immersion in water and what is due to the solute. These tests are effect of: Sucrose, 2,4-dichlorophenoxyacetic acid and indoleacetic acid.

EFFECT OF BLADE TISSUE

To determine how much of the blade must be present on the explant to produce an inhibition of abscission, an experiment was performed in which portions of the blade were excised with the explant. Essentially, it was conducted with explants to which part or all of the blade tissue was still attached. The portions of the blade remaining attached in the various treatments were: A. None (usual explant). B. The basal 10% of the blade. C. The basal 25% of the blade. D. The basal 50% of the blade. E. The entire blade. The material was placed on agar in Petri dishes and examined daily. The results are presented in figure 4. They show a high correlation between the percentages of inhibition of abscission and the amount of the blade tissue present.

EFFECT OF SUCROSE

It has been reported that flower abscission in tomatoes can be reduced by the use of sprays containing sucrose (**13**). To determine whether simi-

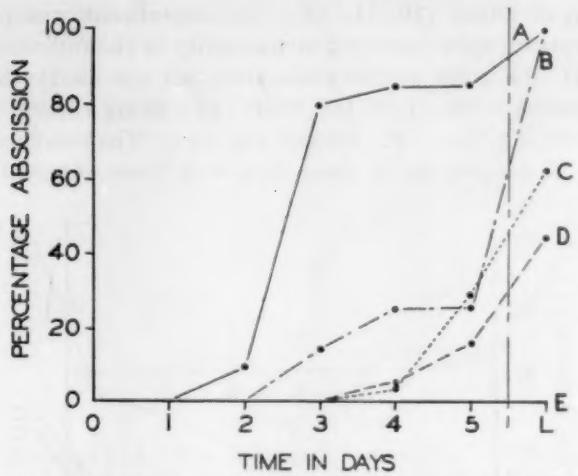

FIG. 4. Effect of blade tissue. Amount of blade present: A. None (usual explant). B. Basal 10% of the blade. C. Basal 25% of the blade. D. Basal 50% of the blade. E. The entire blade.

lar results could be obtained with citrus leaves an experiment was performed in which explants were immersed momentarily in sucrose solutions of the following concentrations: A. None (no immersion control). B. 20%. C. 10%. D. 3%. E. 1%. F. 0.3%. The results are presented in figure 5. They show an inhibition of abscission in explants treated with

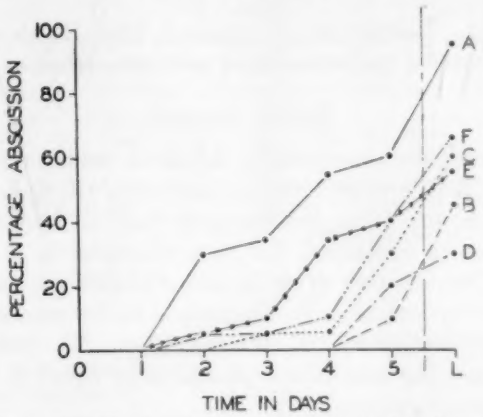

FIG. 5. Effect of dipping in sucrose solutions: A. No dip (control). B. 20% sucrose. C. 10% sucrose. D. 3% sucrose. E. 1% sucrose. F. 0.3% sucrose.

sucrose but there seems to be no particular correlation between the concentration of sucrose employed and the amount of inhibition of abscission.

EFFECT OF 2,4-D

Stewart and his collaborators have worked extensively with 2,4-D (2,4-dichlorophenoxyacetic acid) as an agent for the control of leaf fall and pre-

harvest drop of citrus (**10, 11, 12**). An experiment was performed in which the explants were immersed momentarily in the following concentrations of 2,4-D (the water soluble ammonium salt was used): A. None (no immersion control). B. 1,000 mg./liter. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter. The results are presented in figure 6. A comparison of these data with those obtained following a

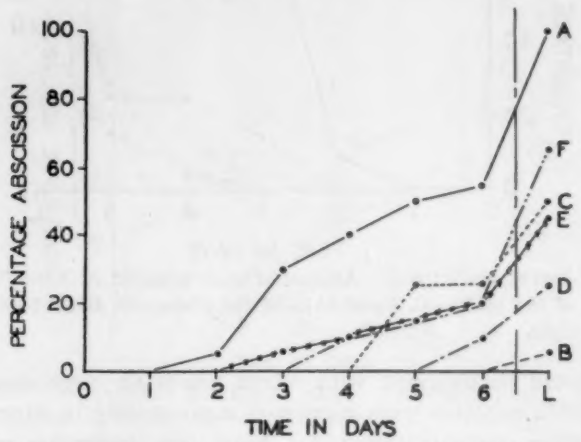

FIG. 6. Effect of 2,4-D. Concentrations of the initial dip: A. No dip (control). B. 1,000 mg./liter. C. 10.0 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter.

short immersion in distilled water (figure 3, Line B) shows that even the lowest concentration of 2,4-D had some inhibitory effect on abscission.

EFFECT OF IAA

Previous investigators have shown that IAA (indoleacetic acid) inhibits abscission under field and greenhouse conditions (**3, 4, 5, 7**). An experiment similar to the preceding one was performed to determine the effect of IAA on abscission in explants. The concentrations in which the explants were immersed immediately prior to being placed on the agar were: A. None (no immersion control). B. Aqueous solution saturated at room temperature. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter. The results are presented in figure 7. Abscission was inhibited at all concentrations.

ABNORMAL GROWTH RESPONSES

Early in this investigation it was noted that there was an unusual enlargement of the parenchyma proximal to the abscission groove after certain treatments. The enlargement was mainly internal since the epidermis was frequently split in the process. The phenomenon was observed with considerable frequency, and while no quantitative measurements were made, a brief description may be of interest.

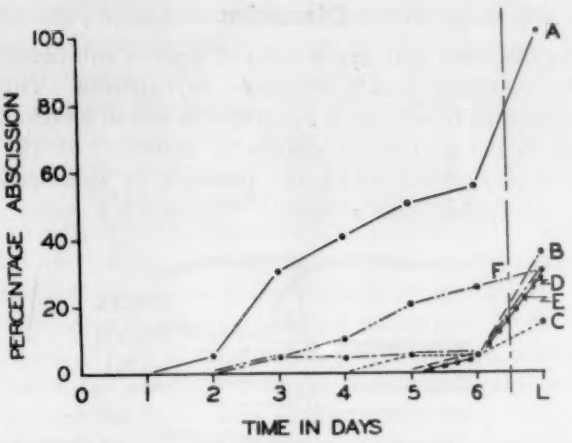

FIG. 7. Effect of IAA. Concentrations of initial dip: A. No dip (control). B. Saturated aqueous solution. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter.

Table I presents a list of conditions which produced this growth response. There is a close correlation between abnormal enlargement and inhibition of abscission. The only exceptions to this correlation were found in the higher concentrations of sucrose, where, it appears very likely, the osmotic factor inhibited cellular activity generally, as well as abscission specifically.

TABLE I

LIST OF EXPLANT TREATMENTS AND PERCENTAGES OF EXPLANTS SHOWING ABNORMAL GROWTH PROXIMAL TO THE ABSCISSION GROOVE

TREATMENT	PERCENTAGE SHOWING GROWTH	TREATMENT	PERCENTAGE SHOWING GROWTH
Immersion in water		Dipped in 2,4-D Solution	
24 hours	65	.00001 mg/L	25
4 hours	25	.001 mg/L	25
½ hour	25	.1 mg/L	25
0 hours	0	10. mg/L	30
		1000. mg/L	25
		Control (No dip)	10
Dipped in Indoleacetic acid solution		Effect of blade	
.00001 mg/L	15	Entire blade	100
.001 mg/L	10	½ blade	55
.1 mg/L	5	¼ blade	?
10. mg/L	0	1/10 blade	10
100. mg/L	10		
1000. mg/L	10	Dipped in sugar solution	
		20% sucrose	0
		10% sucrose	0
		3% sucrose	30
		1% sucrose	15
		.3% sucrose	5

Discussion

It has been observed that application of growth substances, sugars, etc. is followed by localized growth responses in explants. This observation raises the question as to why such overgrowths are so localized. A possible answer, based on the anatomical studies by SCOTT, *et al.* (9) is suggested here. These authors have shown the presence of suberized cells in the vicinity of the abscission zones of citrus. Figure 8 is a drawing showing the

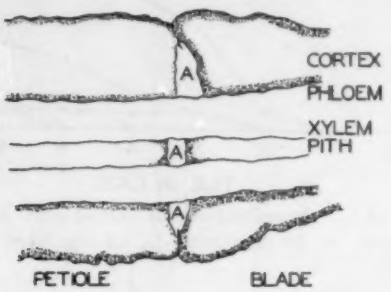

FIG. 8. Diagram of a median longitudinal section thru the Laminar abscission zone. Stipling indicates the extent and degree of tissue suberization. (A) indicates the abscission zone.

location of these suberized cells (stippled area) and their relationship to the abscission zone (A).

The suberized cells are modified parenchyma cells in the pith and in that part of the cortex in and near the bundle sheath, forming a layer two or three cells thick distal to the abscission zone. Proximal to the abscission zone this layer is not quite as thick and the deposit of suberin in the cortical cells adjacent to the abscission zone is light and discontinuous.

This deposition of suberin may be closely related to the restriction of the effects of normal and applied growth substances to the abscission zone. If the applied substances are carried in the normal translocation channel (phloem) there is little chance of their diffusing in large amounts into the surrounding tissues of the blade or petiole, this being prevented by the suberization of the bundle sheath and adjacent tissues in this region. Due to the absence of the bundle sheath at the abscission zone there is no barrier to lateral translocation. Since the suberin deposit of the parenchyma cells proximal, but adjacent, to the abscission zone is discontinuous the diffusing substances can pass into the cortical tissue of the petiole adjacent to the abscission zone. It is obvious that the suberized layer is not completely impermeable to the lateral transport of materials since the deposit is present while the leaf is still in vigorous vegetative condition. It has been suggested by Scott (personal communication) that the transport through the suberized cell walls is through the plasmodesmata. The suberization then would not completely stop the lateral translocation. However, the lateral translocation at the unsuberized abscission zone would be greater than elsewhere.

Many substances, such as sulphur, ammonium thiocyanate, zinc chloride,

chloral hydrate, etc., have been shown to promote abscission in the field. Since these toxic substances differ widely in their chemical properties it is probable that their primary physiological effects are quite different. How does their common abscission-accelerating action come about? It has been reported that the application of an overdose of commercial defoliant to cotton plants results in death of the whole plant, and consequently in no foliar abscission. This indicates that the substances have a toxic action, inactivating the cells of the leaf and thereby producing the same results as artificial deblading. In this investigation the treatment of explants with a commercial defoliant or chloral hydrate inhibited abscission. This inhibition was interpreted as due to the result of death or injury of the cells in the abscission zone. If all of these substances bring about death or injury of the blade tissue, the resultant removal of the inhibitory action of these tissues could account for their effect as promoters of abscission in the field.

The role of ethylene as an accelerator of abscission may be due to its destroying action on auxin. MICHENER (**6**) has suggested that this destruction may possibly occur in intact pea plants. From the present and other investigations, it appears that abscission occurs when a source of growth-promoting substance is not available. Therefore, the possibility that the effect of ethylene in increasing the rate of abscission is one of destruction of growth substances seems reasonable.

It was found in this investigation that abscission is lower than normal in explants immersed in distilled water and then placed on agar plates. Among several possible explanations of this effect are:

First, some substance necessary to abscission may be leached from the explant by the water.

Second, the extra water taken up may release additional abscission inhibitor present in the tissues in a bound state.

Third, the presence of the extra water taken up by the explant during immersion may prevent some metabolic reaction or set of reactions which are necessary for the initiation of the process of abscission.

In the present state of these investigations it is impossible to say which of the above concepts are factors in the delay following immersion. It is hoped that further experiments will clarify this point.

The abscission-inhibiting effect of the growth substances 2,4-D and indoleacetic acid on explants has been exhibited in these tests. However, an analysis of the data (figs. 6 and 7) will show that there is no significant difference between the various concentrations employed.

Summary

1. Abscission in the excised laminar abscission layer of the Valencia orange was studied.

2. Experiments were conducted with explants to determine the effects of: various fractions of blade tissue, immersion in distilled water, sucrose, chloral hydrate, indoleacetic acid, 2,4-dichlorophenoxyacetic acid, ethylene

and cyanamid. Ethylene accelerated abscission, all other treatments inhibited abscission.

3. Many of the treatments brought about enlargement of the tissues proximal to the abscission groove. It is suggested that this may have been the result of interference with free movement of materials by the heavily suberized cells which form a layer surrounding the vascular tissues in the inner cortex and another just distal to the abscission zone in the cortical tissues.

4. This investigation has demonstrated the value of studying abscission in the laboratory using excised tissues of relatively small volume. This method is convenient and the results are comparable to those obtained in the field.

The writer wishes to thank Dr. F. T. Addicott, Dr. F. M. Scott and Dr. R. S. Lynch for their guidance and assistance during this investigation. Thanks are also extended to Dr. S. H. Cameron for the citrus leaves and to E. I. du Pont de Nemours and Company for the 2,4-dichlorophenoxyacetic acid.

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INHIBITION OF NITRATE ASSIMILATION IN EXCISED WHEAT ROOTS BY VARIOUS RESPIRATORY POISONS

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Received December 12, 1949

It is generally recognized that chemical inhibitors of enzymes are highly valuable tools in studies of the intermediary metabolism of various compounds in animals and plants. Such poisons have not been used as extensively in plant studies as their worth in animal studies would justify, and this is particularly so with reference to the problem of nitrate assimilation. Formation of amino acids and other nitrogenous compounds from nitrates must take place in a number of steps. Characterization of this complex series of reactions would be considerably simplified if one were able to block selectively one on another of the enzymes involved and cause an accumulation of intermediate products. It is recognized that few inhibitors appear to be specific for a single enzyme, however complete specificity is evidently not essential for an inhibitor to cause the accumulation of an intermediate. A number of non-specific inhibitors, characterized as "selective" inhibitors by KREBS (**16**) have been found to promote accumulation of various intermediates, and are commonly used.

In a previous paper, NANCE (**20**) reported that nitrate accumulated by wheat roots under aerobic conditions can be assimilated later in an anaerobic environment. The assimilation of previously accumulated nitrate is, in fact, a good deal more active under anaerobic conditions than it is in the presence of oxygen. It appeared, then, that a study of the effects of certain inhibitors on the assimilation of previously accumulated nitrate under anaerobic conditions would be desirable since the effects of these inhibitors on enzymes concerned with the utilization of aerobic oxygen would be eliminated.

The only use of a common respiratory inhibitor on a nitrate reducing system of a higher plant found was that made by BERNHEIM (**5**). He observed that potato aldehyde oxidase preparations are sensitive to cyanide. No reference to the effect of cyanide on the reduction of nitrate in the intact tissue was made. ANDERSON (**2**) tested a number of plants for aldehyde oxidase and found that only 20% of these had such activity. There is certainly insufficient evidence to decide whether or not aldehyde oxidase is part of the nitrate assimilating system of plants generally. A very recent note by BURSTRÖM (**9**) reports the inhibitory effect of n-diamylacetic acid on nitrate assimilation in wheat roots. A more detailed description of these experiments is to appear later.

Experiments by BURSTRÖM (**7**) revealed inhibitory effects on nitrate assimilation in wheat roots by various salts of the alkaline earth, alkali, and heavy metal elements (with the exception of Manganese). NANCE

(**20**) suggested that the inhibitions described by Burström are due to the stimulation of the oxygen inhibition of nitrate assimilation by the salts used.

SHIVE (**22**) grew soybeans at four levels of oxygen tension and discovered that at the higher levels of oxygen the plants did not utilize the nitrate supplied by the culture solution. NANCE (**20**) demonstrated the oxygen inhibition of nitrate assimilation in wheat roots which had been allowed to accumulate nitrate prior to the experimental period and which were in a nitrate-free solution during the experiment.

Greater use has been made of inhibitors in work on the nitrogen metabolism of lower plants. STICKLAND (**27**) investigated the effects of cyanide, carbon monoxide, and oxygen on the reduction of nitrate by *Escherichia coli*. He found that cyanide and oxygen inhibited the reduction, but that carbon monoxide had no effect. QUASTEL (**23**) reported a small carbon monoxide inhibition of nitrate reduction in the same organism. In view of the cyanide, oxygen, and carbon monoxide sensitivity of the nitrate system he proposed that the iron porphyrin enzymes, which are known to be sensitive to these poisons, are directly concerned with nitrate reduction by *E. coli*.

AUBEL (**4**) also working with *E. coli* observed that the reduction of nitrate to nitrite is inhibited both by cyanide and iodoacetate. He reported in a later paper (**3**) that nitrite assimilation in the same organism is also inhibited by these two poisons. WARBURG and NEGELEIN (**30**) demonstrated the cyanide sensitivity of nitrate reduction in Chlorella and suggested that this might be interpreted as evidence of the participation of a heavy metal in the process.

As indicated above, an inhibitor is particularly useful where it can cause the accumulation of an intermediate product of a chain of reactions. There is good evidence for the occurrence of nitrite as an intermediate product in the assimilation of nitrate by higher plants, though the evidence for other suggested intermediates, hydroxylamine, for example, is questionable. ECKERSON (**11**) and HAMNER (**14**) supplied nitrate salts to nitrogen-deficient tomato plants and found by microchemical tests that nitrites appeared in the leaves about eight hours later. DITTRICH (**10**) reported relatively high concentrations of nitrites in shaded plants of *Sambucus sp.* and *Vicia faba*. VIRTANEN (**29**) observed the appearance of nitrite and hydroxylamine in oat and pea seedlings 24 hours after they had been placed in 2 or 4% KNO_3 solutions. The demonstration of nitrite production by the reducase test method of ECKERSON (**11**) is of very doubtful value with reference to the metabolism of higher plants according to BURSTRÖM (**8**) because of the high probability of bacterial activity in the test mixture. The reducase test has been commonly run over a period of from 24 to 48 hours, at relatively high temperature (above 40° C) and with only toluene to prevent bacterial growth. BURSTRÖM (**8**) has questioned the effectiveness of toluene as an agent for the complete elimination of bacterial growth. JONES, SHEPARDSON, and PETERS (**15**) grew soybeans

in culture solutions from which oxygen was excluded by a layer of mineral oil, and found an appreciable accumulation of nitrite in the culture solution. Four days after manganese additions were made to the soybean cultures, no nitrite was found in the solutions. Evaluation of these results is difficult because the effect of the manganese was tested under non-sterile conditions over an extended period of time. It is quite likely that the manganese altered the metabolism of the roots, but one cannot be certain that the manganese did not affect the metabolism of the (probably) large bacterial population.

Materials and methods

Excised roots of four- or five-day-old wheat seedlings of the White Federation variety were used exclusively. There are certain important differences in procedure in the several experiments, however, which will be indicated. The experiments of this paper can be grouped into three categories with reference to methods of tissue preparation and chemical analysis. The earlier experiments were performed with sterile root material following a procedure described in a previous paper (NANCE, 20). These experiments are concerned with the effects of cyanide and carbon monoxide on the complete assimilation of previously accumulated nitrate. Two groups of experiments were performed with non-sterile root material after it was found that the need for the time-consuming procedures of sterile technique could be eliminated if the experimental period were reduced to a period of six hours or less. All but one of the experiments with non-sterile root material were conducted with experimental periods of four hours. One of these two groups of experiments is concerned with the effects of azide and iodoacetate on the complete assimilation of accumulated nitrate. The last series of studies is on the influence of several poisons on the reduction of nitrate to nitrite.

Sterile root material was grown in Petri dishes as described in the earlier paper (NANCE, **20**). Sterile KNO_3 solution (0.005N) was added to each flask and the flasks were shaken at 28° C for four hours. The nitrate solution was then poured off and the roots washed several times with sterile water. Experimental solutions were added, and the flasks, now fitted with rubber stoppers having inlet and oulet tubes, were provided with an atmosphere of nitrogen, or as in one experiment, with carbon monoxide. The flasks were then returned to the shaking apparatus where they remained for 12 hours, again at 28° C.

Non-sterile roots were obtained from wheat seedlings grown in two liter Pyrex crystallizing dishes, the seedlings supported on Fiberglas gauze stretched tightly across the top of the vessel and held in place by rubber bands. For all of the experiments on the formation of nitrite, the seedlings were grown in glass redistilled water. Roots used in the experiments on the effects of azide and iodoacetate on the complete assimilation of nitrate were grown in distilled water half saturated with $CaSO_4$. Use of this salt was suggested by the work of MACHLIS (**17**) who found that the toxic

effects of ordinary distilled water could be eliminated in this fashion. Aeration of the roots was provided by four capillary tubes placed in the vessel.

For one group of experiments with non-sterile root material, the azide and iodoacetate studies, the roots were cut into 15 mm. segments. Tip sections and sections cut from above the 15 mm. mark were kept separate so that a uniform proportion of tip sections and upper sections could be maintained. The root sections were washed several times with water and then placed in 0.01 M KNO_3 for 60 minutes at 25° C aerated by means of shaking. After this, the roots were washed thoroughly again, and counted out into the experimental flasks with 20 tip sections and 25 upper sections per flask. Experimental solutions were added, and the flasks, which were fitted with stoppers having inlet and outlet tubes and connected in a train by means of rubber tubing, were provided with an atmosphere of nitrogen by passing the purified gas through the flasks for 15 minutes. The flasks were clamped off at that time and placed in a water bath maintained at 25° C. All flasks were shaken during the experimental period. All experimental solutions were adjusted to pH 6.0, with the exception of a few indicated cases.

For the experiments on the reduction of nitrate to nitrite, KNO_3 was added to the redistilled water in which the seedlings were grown to give a concentration of 0.005 M, 12 to 18 hours before the roots were excised. The roots were then cut from the seedling at a point 45 mm. from the tip, and these tip sections were used exclusively. After the tips were washed six or more times with distilled water, 24 tips were placed in each flask. From this point on, the experimental procedure followed that just described above for the experiments on the effects of azide and iodoacetate on the complete assimilation of nitrate.

To determine the quantity of nitrate completely assimilated, the amount of nitrate remaining in the tissue at the end of the experimental period ("final" nitrate concentration) was subtracted from the amount of nitrate found in a sample taken at the end of the period of accumulation ("initial" nitrate concentration). This difference is simply a measure of nitrate disappearance and one cannot be certain that it represents it its entirety nitrate completely converted to various amino compounds or to other organic compounds having nitrogen atoms of like oxidation level. Since the phenoldisulfonic acid method includes both nitrate and nitrite ions, nitrate reduced only to the level of nitrite will not contribute to the difference between the initial and final nitrate concentrations. Because other intermediates have not been positively identified, nothing definite can be said about their fate in the phenoldisulfonic acid method. It is possible that some nitrate is converted to gaseous nitrogen. This effect would, of course, contribute to the apparent nitrate assimilation. Estimation of the quantity of nitrate assimilated by difference is rather commonly used, as for example by BURSTRÖM (**6**–**9**). In order to distinguish nitrate reduced to nitrite

from nitrate disappearance as measured by the phenoldisulfonic acid method, the latter is referred to in the present paper as nitrate "completely assimilated."

Analysis for nitrate followed a procedure adapted from BURSTRÖM (**6**) described in the earlier paper by NANCE (**20**). The determination was made by the phenoldisulfonic acid method on a hot water extract of the roots. In some experiments the analysis was made on the root extract and experimental solution separately, and in other cases on the combined root extract and experimental solution.

It was found that no nitrite appeared in the roots immediately after the period of accumulation, so the amount of that anion produced under the experimental conditions could be determined directly. Because of the high reactivity of the nitrite ion, it was felt that extraction with hot water might involve important losses of that anion. For this reason a very simple procedure was used. Each 50 ml. flask with its 5 ml. of experimental solution and root material was stoppered and placed in the freezing chamber of a refrigerator immediately following the termination of the experiment. The contents of the flasks were frozen solidly in less than an hour. On the following day the contents of the flasks were allowed to thaw at room temperature. The liquid in the flasks was then made to 10 ml., poured back into the flasks and shaken vigorously. After about 15 minutes a 5 ml. aliquot was pipetted from each flask for analysis. Nitrite analyses were made by the Griess-Ilosvay colorimetric method as given by SNELL and SNELL (**25**) on combined root extract and experimental solution. It was found that with this relatively crude method, nitrite analyses on root samples subjected to duplicate experimental treatment agreed within 5%. It is recognized that this method can only be relied on at present for comparative purposes.

Analytical results in the experiments on carbon monoxide and cyanide are expressed in terms of the dry weights of the roots. In the other experiments the results are simply given in terms of the number of roots per sample.

Except for the studies on carbon monoxide, the roots were in an atmosphere of nitrogen during the experimental period. In the earlier experiments unpurified tank nitrogen was used. These experiments, which were all done under sterile conditions, ran for periods of 12 hours or more so that it is quite likely that all of the oxygen in the flasks was consumed during the experiment. In all other experiments the nitrogen was passed through an alkaline solution of sodium anthraquinone b-sulfonate and sodium hydrosulfite, an oxygen absorbant described by PETERS and VAN SLYKE (**21**).

Results

EFFECTS OF INHIBITORS ON THE COMPLETE ASSIMILATION OF NITRATE

Carbon monoxide appeared to be a poison of some interest in this study since participation of iron porphyrin enzymes in nitrate reduction has been

suggested by several investigators (**23, 30**). The roots were in an atmosphere of pure carbon monoxide, prepared by addition of concentrated sulphuric acid to formic acid. During the experimental period the roots were in the dark. It is evident that carbon monoxide does not affect the assimilation of accumulated nitrate (table I).

TABLE I

THE EFFECT OF CARBON MONOXIDE ON THE ASSIMILATION OF ACCUMULATED NITRATE

EXPERIMENTAL TREATMENT†	NITRATE IN mM PER GRAM DRY WEIGHT		
	INITIAL*	FINAL	REDUCED
N_2 atmosphere	0.35	0.18	0.17
CO atmosphere	0.35	0.16	0.19

* Accumulated by a representative sample of four hours from an aerated solution of 0.005 M KNO_3 and 0.01 M sucrose.
† Roots in 0.01 M sucrose during experimental period.

The first experiment with cyanide indicated that this poison is a strong inhibitor of nitrate assimilation, but there was a serious lack of uniformity with duplicate treatments. In later experiments it was found that control of the hydrogen ion concentration of the experimental solution by means of phosphate buffer yielded replicate results agreeing within 4%. Such an experiment is reported in table II. In the earlier experiments (NANCE, **18**)

TABLE II

INHIBITION OF THE ASSIMILATION OF ACCUMULATED NITRATE BY KCN UNDER ANAEROBIC CONDITIONS

EXPERIMENTAL TREATMENT†	INITIAL*	NITRATE IN mM PER GRAM DRY WEIGHT		
		FINAL		REDUCED
		IN ROOTS	LOST	
KCN 0.001M, K-Na phosphate buffer pH 5.4	0.61	0.15	0.46	0
KCN 0.001M, K-Na phosphate buffer pH 6.7	0.61	0.33	0.25	0
K-Na phosphate buffer pH 5.4	0.61	0.31	0	0.30
K-Na phosphate buffer pH 6.7	0.61	0.38	0	0.23

* Accumulated by a representative sample in four hours from an aerated solution of 0.005M KNO_3 and 0.01M sucrose.
† All solutions contain 0.01M sucrose.

where no respiratory poisons were used there were no losses of nitrate, within the limits of error of the method, from the roots to the external solution during the time the roots were under nitrogen. The cyanide toxicity, however, evidently causes a loss of nitrate to the experimental solution which is greater at the higher concentrations of hydrogen ion.

Another experiment (table III) indicates the persistence of the inhibitory effect of the cyanide. After the roots had accumulated nitrate for

TABLE III

INHIBITION OF THE ASSIMILATION OF ACCUMULATED NITRATE FOLLOWING A LIMITED EXPOSURE TO CYANIDE

EXPERIMENTAL TREATMENT INTERMEDIATE SOLN.†	pH OF FINAL SOLN.†	NITRATE IN MM PER GRAM DRY WEIGHT			
		INITIAL*	FINAL		REDUCED
			IN ROOTS	LOST	
KCN 0.001M Buffer pH 4.5	Buffer pH 4.5	0.82	0.45	0.34	0.00
KCN 0.001M Buffer pH 6.6	Buffer pH 6.6	0.82	0.68	0.13	0.00
KCN 0.001M Buffer pH 8.8	Buffer pH 8.8	0.82	0.71	0.10	0.00
K_2SO_4 0.001M Buffer pH 6.6	Buffer pH 6.6	0.82	0.61	0.00	0.21

* Accumulated by a representative sample in four hours from an aerated solution of 0.005M KNO_3 and 0.01M sucrose.

† All solutions contain sucrose 0.01M and K-Na phosphate buffer of the pH indicated.

four hours, they were placed in cyanide solutions of various concentrations with phosphate buffer, and aerated in these "intermediate" solutions for an hour. The roots were then washed and placed in solutions containing only 0.01 M sucrose, and, as in the previous experiment, kept under nitrogen for 12 hours. The inhibition of nitrate assimilation was still complete but the injury to the roots as measured by the loss of nitrate was diminished.

Experience with cyanide indicated the necessity of analysis of both the experimental solutions and the root extracts for nitrate. In the experiments on azide and iodoacetate the root extracts and experimental solutions were combined and analyzed as one sample. It was quickly discovered, however, that this technique was not feasible with the analytical method used. Where iodoacetate was present, the combined root extract and experimental solution on analysis often appeared to have no nitrate at all. Analysis of known samples consisting of nitrate plus iodoacetate, indicated that the poison causes a disappearance of nitrate. It has been known for some time that chlorides interfere with the determination of nitrate by the phenol-disulfonic acid method as adapted for analysis of plant tissue. Chlorides cause losses of nitrate and this is thought to involve the formation of nitrosyl chloride. It seems likely that the effect of iodoacetate may be a related effect associated with iodide.

Analysis of the combined root extract and external solution in the presence of sodium azide yielded amounts of nitrate far in excess of that known to be present in the roots at the end of the period of accumulation. This

certainly appears to be due to an oxidation of the azide to nitrate by the hydrogen peroxide which is used to oxidize interfering organic material.

To avoid these analytical difficulties with azide and iodoacetate, the technique used in the second experiment with cyanide reported above was followed with some modification. The root sections were allowed to accumulate nitrate from an aerated solution of 0.01 M KNO_3 for an hour. The roots were then washed, counted out into experimental flasks and intermediate solutions of 0.0005 M Na_2SO_4 (the control), 0.001 M NaN_3, and 0.001 M $CH_2ICOONa$ were added. After one hour these solutions were poured off and the roots were washed with distilled water. Root samples from each treatment were then set aside for determination of the initial nitrate levels. Other samples from each treatment were placed in 0.025 M KH_2PO_4 in an atmosphere of nitrogen for six hours at 25° C. These were then analyzed for the final nitrate levels. The phosphate solution (pH about 4.5) was used because in other experiments it had been found to overcome the mildly inhibitory effects of various neutral salts. It was felt that this procedure would have two advantages: the roots to be analyzed for the initial nitrate content would have the same level of poison as those placed under anaerobic conditions and the level of poison finally appearing in the combined root extract and experimental solution would be sufficiently low that the effect on the analysis would be small or even insignificant. The results of this experiment appear to confirm these expectations (table IV). There is a marked inhibition both by azide and iodoacetate.

TABLE IV

INHIBITION OF THE ASSIMILATION OF ACCUMULATED NITRATE BY AZIDE AND IODOACETATE

EXPERIMENTAL TREATMENT†	NITRATE IN mM × 10–4 PER 45 ROOT LENGTHS			
	INITIAL	FINAL*	REDUCED	PERCENTAGE REDUCED
Na_2SO_4 0.0005M	9.50	5.45	4.55	47
NaN_3 0.001M	7.85	5.95	1.90	24
$CH_2ICOONa$ 0.001M	7.25	5.60	1.65	23

* Nitrate in combined external solution and root extract after six hours in 0.025 M KH_2PO_4 in an atmosphere of pure nitrogen.

† Roots were in KNO_3 0.01 M for one hour and were then transferred to the experimental solutions for one hour. All these solutions were aerated.

EFFECTS OF INHIBITORS ON THE REDUCTION OF NITRATE TO NITRITE

Since the complete assimilation of nitrate is, obviously, a process involving a number of enzyme systems, studies of the type reported above are rather difficult to interpret. It seemed that studies of the complete assimilation of nitrate might be profitably abandoned in favor of work on certain steps in the process mediated by one or perhaps only a few enzymes. By far the most obvious choice for such a study is the nitrate-nitrite system. Nitrite accumulation under anaerobic conditions is commonly observed in

a number of lower plants. These results suggested the procedure which was employed in the following experiments.

Roots which had been allowed to accumulate nitrate from 0.005 M KNO_3 for periods of from 12 to 18 hours were placed in an atmosphere of purified nitrogen for periods up to four hours at 25° C. Nitrites were produced during this anaerobic period in most experiments. In a few experiments, no nitrite could be found in the combined root extract and experimental solution. These failures appear to be due to incomplete removal of the traces of oxygen that are to be found in commercial nitrogen of even the high purity grade.

BURSTRÖM (**8**) has properly pointed out the possibility of bacterial production of nitrite in such an experiment. In all the experiments on nitrite formation reported in this paper, the roots were under anaerobic conditions for not more than four hours. In none of the experiments of this duration was there a development of a discernible cloudiness. Several experiments were run in which the quantity of nitrite formed at various time intervals was determined. In all of these experiments it was found that the rate of nitrite production was not significantly altered over a period of four hours.

One would expect an increasing rate of nitrite formation if microorganisms were responsible for the reduction of the nitrate. There is however, the rather remote possibility that the residue of bacterial cells on the surfaces of the roots, after the roots were washed six or more times with distilled water, could have produced the nitrite. This possibility was tested in two trials with sterile roots grown as described in the earlier paper (**20**). In both of these trials, the sterile roots placed in 0.01 M KNO_3 under anaerobic conditions produced nitrite in quantities of the order of magnitude of that produced by non-sterile roots containing previously accumulated nitrate.

Several experiments with cyanide, one of which is reported in table V revealed the toxic effects of that poison on the reduction of accumulated

TABLE V

INHIBITION OF NITRITE APPEARANCE BY POTASSIUM CYANIDE

KCN CONCENTRATION IN MOLES/LITER	NITRITE IN $mM \times 10^{-4}$ PER 24 ROOT TIPS	PERCENTAGE OF CONTROL
None (control)	0.27	100
1×10^{-5}	0.33	123
1×10^{-4}	0.05	18
1×10^{-3}	0.00	0

nitrate to nitrite. In all of the experiments the small stimulation of nitrite formation at concentration of 10–5 M was seen. As in the experiments on the complete assimilation of nitrate, the inhibition was absolute at 0.001 M.

Iodoacetate appears to be quite an effective inhibitor of nitrite formation

TABLE VI

INHIBITION OF NITRITE APPEARANCE BY IODOACETATE

CH_2ICOON_a IN MOLES/LITER	NITRITE IN $mM \times 10^{-4}$ PER 24 ROOT TIPS	PERCENTAGE OF CONTROL
None (control)	0.36	100
1×10^{-6}	0.33	95
1×10^{-5}	0.20	57
1×10^{-4}	0.12	34
1×10^{-3}	0.08	23

(Table VI). Unlike cyanide, iodoacetate does not stimulate nitrite formation at any concentration.

Sodium azide was tried in one experiment and it appeared to inhibit the formation of nitrite quite effectively. When all the inhibitors used in this study were tested for their effect on the nitrite determination, only azide interfered. Reference is made to the work of FRIEND (**12**), where it is stated that nitrite and azide react to form nitrogen gas, nitrous oxide, and water.

Discussion

Oxygen appears to be one of the most effective inhibitors of nitrate assimilation, under certain conditions at least (**20, 24**). Nitrate assimilation, however, does not seem to be inhibited by oxygen. When supplied to plants in the culture solution with adequate aeration, nitrite nitrogen is assimilated as rapidly as it is absorbed, so long as the roots have a source of respirable material (MEVIUS and DIKUSSAR, **19**). After the depletion of this food material there may be a temporary accumulation of nitrite, but shortly after this occurs, the tissues die. Evidence discussed below indicates that nitrite assimilation is stimulated by respiratory activity, in other words, the process requires oxygen.

It is well known from the classical experiments of Prianischnikov and his students (**22**), that ammonium ions are likewise immediately assimilated when adequate carbohydrate reserves are available, and that depletion of the carbohydrate is followed by the death of the tissues. Nitrates, unlike other forms of inorganic nitrogen available to the plant, can be stored as such (MCCALLA, **18**). In other words, the plant can control the assimilation of the nitrate. It appears from the present work and that published earlier (**20**), that the control of nitrate assimilation in the plant is effected by the oxygen inhibition of the reduction of nitrate to nitrite. The mechanism of this oxygen inhibition is not obvious but its similarity to the oxygen inhibition of fermentation is suggestive.

The complete lack of sensitivity of nitrate assimilation to carbon monoxide under anaerobic conditions appears to dispose of the possibility that enzymes of the cytochrome system and other oxidases affected by that gas are directly involved in nitrate assimilation.

Cyanide and azide are also very effective inhibitors of the cytochrome system, and in view of the carbon monoxide results it is necessary to consider other possible systems that may be concerned in the inhibition of nitrate assimilation by cyanide and azide under anaerobic conditions. Cyanide has been reported to inhibit certain oxidases other than cytochrome oxidase, several dehydrogenases, an amidase and a decarboxylase (SUMNER and SOMERS, **28**). The sensitivity of potato aldehyde oxidase to cyanide, demonstrated by BERNHEIM (**5**), has been mentioned. It seems most likely that cyanide inhibits the assimilation of nitrate in wheat roots at the nitrate-nitrite level, though the possibility of an effect at some other level is certainly not eliminated.

Because of the reaction between azide and nitrite it is difficult to determine the effect of this poison in the reduction of nitrate to nitrite. There is some reason to believe, nevertheless, that azide may affect the nitrate-nitrite system. The reaction between azide and nitrite would promote a disappearance of nitrate if there were no other azide effect. It was found, however, that the disappearance of nitrate is blocked by azide. It is possible, then, that azide inhibition involves some property of the poison other than its ability to react with nitrite. As with cyanide, it is likely that azide blocks the reduction of nitrate to nitrite. If it happens that the enzymes of the root can metabolize nitrite in some fashion that does not permit the azide to react with the nitrite, the poison could produce its effect in still another manner. Azide has been shown to uncouple oxidation and phosphorylation in yeast by SPIEGELMAN, SUSSMAN and KAMEN (**26**). In cells treated with azide the chemical energy released in the oxidation of phosphoglyceraldehyde is not available for the uses that the cell may put it to including, of course, various syntheses.

Iodoacetate is known to inhibit a number of dehydrogenases (**28**) including one of particular importance in the oxidation of carbohydrate, triose phosphate dehydrogenase (ADLER *et al.* **1**). It is quite probable that nitrate reduction in wheat roots is coupled with dehydrogenase activity much as nitratase preparations made from *E. coli* which have been demonstrated by GREEN and co-workers (**13**) to accept hydrogen from a number of dehydrogenases in the presence of suitable carriers.

All of the poisons used in this study appear to inhibit nitrite formation by their effects on the enzyme or enzymes concerned with the reduction of nitrate to nitrite. It should be pointed out, however, that the quantity of nitrite accumulated under various conditions can be affected in other ways. As with any intermediate product, the amount of nitrite that appears is affected both by the rate of formation and by the rate of consumption of the anion. The extreme rarity with which nitrite appears under aerobic conditions is evidently due to the fact that the nitrite is consumed as rapidly as it is formed. Conversely, under anaerobic conditions the rate of consumption is less than the rate of formation. These facts suggest that nitrite consumption is dependent on certain processes that proceed more rapidly

in the presence of oxygen than in its absence. It is possible that these processes are concerned with the metabolism of the organic acids. One must concede that at some stage in the process of nitrate assimilation an inorganic, nitrogenous intermediate must react with some non-nitrogenous, organic intermediate (the familiar "carbon skeleton" of a potential amino acid). Whether this reaction takes place after the nitrate has been reduced to the level of ammonia, the classical idea, or before, it is evident that an adequate supply of the non-nitrogenous intermediate (or intermediates) is required to prevent an accumulation of the inorganic, nitrogenous intermediate (or intermediates) when rapid formation of the latter material is favored. It certainly appears that non-nitrogenous intermediates required in the synthesis of amino acids, at least, can only come from organic acid precursors.

Summary

The effects of several commonly employed respiratory poisons on the complete assimilation of nitrate and the reduction of nitrate to nitrite by the excised roots of wheat seedlings were observed. Carbon monoxide in the dark does not inhibit the assimilation. Cyanide and iodoacetate inhibit both reduction of nitrate to nitrite and the complete assimilation of nitrate. Azide inhibits the complete assimilation of nitrate but its effects on the formation of nitrite cannot be observed directly because of the reaction between azide and nitrite.

The portion of this work done in the Department of Botany at the University of Wisconsin was supported by a grant from the Wisconsin Alumni Research Foundation.

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FLUORINE TOXICITY IN TOMATO AS MODIFIED BY ALTERATIONS IN THE NITROGEN, CALCIUM, AND PHOSPHORUS NUTRITION OF THE PLANT[1]

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Received February 8, 1950

Introduction

With recent advances in the use of fluorine as a raw material in many manufacturing processes, it has become more and more obvious that certain compounds of this element are responsible for injury to crops, native vegetation, and ornamental plantings in the vicinity of such industries. As has been shown with other acid gases (**2, 7, 9**) many factors are involved in determining the susceptibility of plants to the effects of fluorine. In detailed inspections of peach orchards in New Jersey areas which had undergone accidental fluorine fumigations in 1944, it was often observed that trees of the same variety and similarly situated with respect to the source of fluorine failed to show a uniform degree of gas injury. The theory was advanced that such variation might be due, at least in part, to individual differences in growth status. This investigation was conducted to determine the role of nitrogen, calcium, and phosphorus in influencing plant susceptibility to injury from fluorine supplied as NaF in the nutrient substrate or as HF in the atmosphere.

Methods

Tomato (*Lycopersicum esculentum*, Rutgers), chosen as the test plant, was grown in sand culture during the summer of 1948. The tomato seeds were germinated in washed quartz sand and, at the age of two weeks, 96 uniform seedlings were transplanted to chemically purified sand in glazed crocks, two plants per crock, making a total of 48 cultures. These seedlings were flushed daily with 500 ml. of a basic nutrient solution of the following composition: .0010 M $Ca(NO_3)_2$, .0040 M KNO_3, .0010 M $MgSO_4$, .0010 M KH_2PO_4, and .0008 M $(NH_4)_2SO_4$ with trace amounts of B, Mn, Zn, and Fe. Three weeks later the 48 cultures were divided into four series of 12 cultures each. Within each series the nitrogen, calcium, or phosphorus nutrition was altered by varying the concentration of the respective element in the basic nutrient solution. Concentrations of each of the three major elements were selected in a range to include deficient, optimum, and excessive supplies to the plant; *viz.* 14, 56, 112, and 448 p.p.m. N; 10, 40, 80, and 240 p.p.m. Ca; and 0, 0.8, 15.5, and 62 p.p.m. P (table I). Work on tomato nutrition conducted in this laboratory during recent years served as a basis for the selection of optimal levels of the major elements (**1, 4, 6, 8**). Criteria selected as indications of nutritional status included green weight, per-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station.

TABLE I

MOLECULAR COMPOSITION OF NUTRIENT SOLUTIONS* EMPLOYED IN GROWING TOMATO PLANTS

NUTRITIONAL TREATMENT	$Ca(NO_3)_2$	KNO_3	K_2SO_4	$MgSO_4$	KH_2PO_4	$Mg(NO_3)_2$	$(NH_4)_2SO_4$	$CaSO_4$
p.p.m.	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*
N 14	.0004	.....	.0020	.0010	.0010	.....	.0001	.0006
N 56	.0010	.0012	.0014	.0010	.0010	.....	.0004	.....
N 112	.0010	.0040	.....	.0010	.0010	.0002	.0008	.....
N 448	.0010	.0040	.....	.....	.0010	.0098	.0032	.....
Ca 10	.0003	.0040	.....	.0010	.0010	.0008	.0005	.....
Ca 40	.0010	.0040	.....	.0010	.0010	.....	.0005	.....
Ca 80	.0020	.0020	.0010	.0010	.0010	.....	.0005	.....
Ca 240	.0030	.....	.0020	.0010	.0010	.....	.0005	.0030
P 0	.0010	.0040	.0005	.0010	.....	.....	.0005	.....
P 0.8	.0010	.0040	.0005	.0010	.00003	.....	.0005	.....
P 15.5	.0010	.0040	.0003	.0010	.0005	.....	.0005	.....
P 62	.0010	.0030	.....	.0010	.0020	.0005	.0005	.....

* Trace elements were supplied in the following forms: Mn, 0.25 p.p.m. ($MnSO_4$); B, 0.50 p.p.m. (H_3BO_3); Zn, 0.50 p.p.m. ($ZnSO_4$); Fe, 1.00 p.p.m. ($FeSO_4$).

centage dry weight, and type of growth of plant tops, and condition of leaves (table II).

TABLE II

TOTAL GREEN WEIGHT, PERCENTAGE DRY WEIGHT, LEAF COLOR, AND GENERAL GROWTH OBSERVATIONS OF TOPS OF TOMATO PLANTS HAVING VARIED N, CA, AND P NUTRITION AND NO FLUORINE TREATMENT

NUTRIENT TREATMENT	TOTAL GREEN WEIGHT	PERCENTAGE DRY WEIGHT	LEAF COLOR	GROWTH OBSERVATION
p.p.m.	*gms.*	%		
N 14	81.5	13.1	Chlorotic	Spindly
N 56	187.3	11.4	Light Green	Medium hard
N 112	289.8	10.7	Green	Vigorous
N 448	203.1	11.3	Dark Green	Severe Fasciation
Ca 10	112.1	12.0	Chlorotic	Stunted
Ca 40	222.7	10.0	Green	Vigorous
Ca 80	300.7	11.5	Green	Vigorous
Ca 240	278.5	10.4	Dark Green	Vigorous
P 0	207.4	12.1	Purple underside	Spindly
P 0.8	276.4	11.2	Slightly purple	Fairly active
P 15.5	216.9	10.4	Green	Vigorous
P 62	302.8	10.7	Green	Vigorous

When the plants were in a condition reflecting the specific nutrient treatments, each series was subjected to a different fluorine exposure. Series A and B received fluorine in the form of NaF in the nutrient substrate, and series C and D were exposed to HF in a fumigation chamber. In series A, 50 p.p.m. F as NaF were added to the nutrient solutions daily for 13 days, at which time injury was definitely established within each nutritional group. The plants were then harvested. In series B. 50 p.p.m. F as NaF were added to the nutrient solutions daily until the development within each culture of a definite "end-point" of injury consisting of necrosis of the tips and margins of the youngest fully expanded leaves. Each plant was harvested as it reached this "end-point." Those which were not injured were allowed to grow for 31 days. The plants in series C were fumigated in a specially constructed glass-walled chamber through which a mixture of air and HF was continually passed (air exchanged once a minute) at an average concentration of .048 p.p.m. F for four and one half hours. One day later, in the absence of characteristic signs of fluorine injury, one plant from each nutritional treatment was harvested. The remaining plants, still exhibiting no effects of fluorine damage, were harvested seven days later. Series D plants were fumigated at .47 p.p.m. F for three and one half hours. The next day, when fluorine toxicity symptoms were apparent on all plants, one plant from each treatment was harvested. The remaining plants were harvested seven days later.

At harvest, all plants were weighed and separated into leaf and root fractions. The roots were washed as thoroughly as possible with distilled water, and then all samples were placed in a drying oven at 45° C for

24 hours. Dry weights were obtained and the samples were ground through the 40-mesh screen of a semi-micro Wiley mill and analyzed for fluorine by the method described in a previous paper (**3**). Fluorine contents were reported as parts per million of dry weight.

Results and discussion

Response of plants to addition of NaF in the nutrient solution

To compare the relative degree of fluorine toxicity on plants of varying nutritional status, all plants of series A, harvested on the 12th day of exposure to fluorine in the nutrient solution, were examined. The results are compiled in table III. In the nitrogen group, only the 112 p.p.m. N plants

TABLE III

Degree of injury and fluorine content of series A tomato plants having varied N, Ca, and P nutrition and treated for 13 days with 50 p.p.m. F as NaF in the substrate

Nutrient treatment	Degree of injury	P.p.m. F in tissues		Total micrograms F in entire plant	Percentage of total F present in leaves
		Leaves	Roots		
p.p.m.		*p.p.m.*	*p.p.m.*	*micrograms.*	*%*
N 14	None	74	280	1,126	33
N 56	None	96	3010	8,895	9
N 112	Moderate	540	2000	14,000	38
N 448	None	160	1800	9,104	21
Ca 10	None	170	460	2,202	54
Ca 40	Moderate	950	1290	12,216	75
Ca 80	None	116	3610	11,575	9
Ca 240	None	62	4360	15,855	4
P 0	Trace	274	2739	7,067	30
P 0.8	Slight	480	2305	17,612	27
P 15.5	Slight	464	1050	8,278	59
P 62	Moderate	1010	970	12,117	79

showed injury during the 13-day fluorine treatment. This exposure period was obviously too short to cause injury to any of the other plants. The roots of all plants reflected the condition of the foliage. The plants grown at 14 p.p.m. N were generally spindly and hard, and their root systems were correspondingly small and free from signs of fluorine injury. At 56 p.p.m. N the root systems as well as the tops of the plants were larger and also uninjured. The roots of the 112 p.p.m. N plants, however, were dark brown, indicative of severe fluorine injury. The roots of the 448 p.p.m. N plants were slightly brown, probably a result of the excessive nitrogen supply rather than of fluorine injury.

Of the calcium group, the only plants visibly injured after 13 days of root treatment were those grown at 40 p.p.m. Ca. Again root condition was associated with the presence or absence of fluorine injury in the top of the plant. Roots of the 40 p.p.m. Ca plant were badly injured, whereas those of all other plants were normal in appearance.

In the phosphorus group, all plants showed some degree of fluorine injury, which was correlated directly with the amount of phosphorus in the substrate, those at the highest level (62 p.p.m. P) being most severely injured. The condition of the roots was also correlated with fluorine injury to the tops of the plants. Since the basic nutrient solutions used in growing the plants of this group contained the nitrogen and calcium levels most conducive to fluorine toxicity, it is within reason that all plants in the phosphorus group might show some degree of fluorine injury within 13 days. The fact that even the plants at the highest P level, 62 p.p.m., were severely injured might be explained by the failure of the phosphorus range to extend to a concentration beyond the optimum level for active growth. According to this reasoning, 62 p.p.m. P might represent a medium rather than a high phosphorus level. On the other hand, further increase in phosphorus might conceivably continue to accentuate fluorine injury.

Series B, in which the plants were harvested as each reached an "end-point" of injury, indicated that plants in an optimum condition of active growth showed fluorine injury as a result of the addition of NaF to the nutrient substrate within a much shorter time than did those in a less active state of growth. In the group receiving varying amounts of nitrogen prior to and during fluorine treatment (table IV), the 112 p.p.m. N

TABLE IV

DEGREE OF INJURY, NUMBER OF DAYS OF FLUORINE TREATMENT AND FLUORINE CONTENT OF SERIES B TOMATO PLANTS HAVING VARIED N, CA, AND P NUTRITION AND TREATED WITH 50 P.P.M. F AS NAF IN THE SUBSTRATE. ALSO FLUORINE CONTENT OF CONTROL PLANTS.

NUTRIENT TREATMENT		SERIES B				CONTROL	
		DEGREE OF INJURY	NO. OF DAYS	F CONTENT		F CONTENT	
				LEAVES	ROOTS	LEAVES	ROOTS
	p.p.m.			*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
N	14	None	31	250	1310	10	23
N	56	Moderate	24	1000	3440	33	10
N	112	Moderate	13	500	1185	30	23
N	448	None	31	430	1220	20	20
Ca	10	None	31	435	480	15	15
Ca	40	Moderate	6	1000	750	33	13
Ca	80	Moderate	8	850	1300	38	19
Ca	240	None	31	161	3945	20	10
P	0	Trace	31	421	6565	10	12
P	0.8	Slight	31	620	2305	18	10
P	15.5	Moderate	13	890	2080	20	12
P	62	Severe	13	672	1340	15	14

culture exhibited typical "end-point" injury at the earliest date, 13 days after the initial treatment. These plants had been rated as the most vigorous of the nitrogen group. The 56 p.p.m. N culture, which was slightly harder, reached the "end-point" 24 days after the start of treatment.

Although fluorine treatment of the other plants was continued for an entire month, the 14 p.p.m. N plants, which were extremely hard, and the 448 p.p.m. N culture, which was over-vegetative, displayed no signs of fluorine toxicity.

In the group receiving differential calcium nutrition, the plants grown at medium levels, 40 and 80 p.p.m. Ca, were the first to show "end-point" fluorine injury, within six and eight days, respectively. (The 80 p.p.m. Ca culture was uninjured in series A after 13 days). The 10 p.p.m. Ca plants, which were extremely stunted and abnormal as a result of inadequate calcium and the 240 p.p.m. Ca plants at the other extreme of the calcium range showed no fluorine injury throughout the 31-day period of treatment.

As a result of varying the amounts of phosphorus in the nutrient solution, the trend was different from that observed in the nitrogen and calcium groups. Here, plants grown at the two highest concentrations, 15.5 and 62 p.p.m. P, reached the "end-point" of fluorine injury within the shortest time, 13 days. By the end of the 31-day treatment the 0.8 p.p.m. P plants had barely reached this point, and the 0 p.p.m. P plants, which were slightly hard, exhibited just a trace of fluorine injury.

Response of plants to addition of F as HF in the atmosphere

The tomato plants in series C, which were fumigated at the lower concentration of .048 p.p.m. F for four and one half hours, showed no sign of injury to leaves or roots (table V). On the other hand, all series D plants, fumigated at .47 p.p.m. F for three and one half hours, showed varying degrees of fluorine injury one day after fumigation. As with the series receiving fluorine through the roots, fluorine fumigation caused most marked symptoms to the actively growing plants. In the nitrogen group, the 112 p.p.m. N and the 56 p.p.m. N plants were most severely injured.

In the calcium group, leaves of the 40 p.p.m. plants were more severely injured than those of the 80, 10, and 240 p.p.m. plants, and of the latter group, the 10 and 240 p.p.m. plants were the least injured. As in the nitrogen group, roots were uninjured by fluorine fumigation.

As in the phosphorus group treated with fluorine through the roots, the degree of injury in the group subjected to fumigation was directly correlated with the amount of phosphorus the plant had received in the nutrient solution, the leaves of the 62 p.p.m. P plant being most severely injured. The roots of all plants in this group were uninjured.

Seven days after fumigation there was no significant change in the relative degree of injury within each nutritional group. Only a drying out of the burned areas occurred, producing a typical buff coloration (**3**).

Fluorine analysis of tomato plants

Exposed to F as NaF in the nutrient solution

As all plants from series A were harvested after the same period of F treatment, the analytical results are directly comparable. The fluorine

TABLE V

DEGREE OF INJURY* AND FLUORINE CONTENT OF SERIES C AND SERIES D TOMATO PLANTS HAVING VARIED N, CA, AND P NUTRITION AND FUMIGATED AT .048 P.P.M. F FOR FOUR AND ONE HALF HOURS AND AT .47 P.P.M. F. FOR THREE AND ONE HALF HOURS RESPECTIVELY. DUPLICATE PLANTS IN EACH SERIES WERE HARVESTED ONE AND SEVEN DAYS RESPECTIVELY AFTER FUMIGATION.

NUTRIENT TREATMENT	SERIES C P.P.M. F IN TISSUES				DEGREE OF INJURY	SERIES D P.P.M. F IN TISSUES			
	LEAVES		ROOTS			LEAVES		ROOTS	
	1 DAY	7 DAYS	1 DAY	7 DAYS		1 DAY	7 DAYS	1 DAY	7 DAYS
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
N 14	40	53	23	13	Slight	375	415	20	18
N 56	43	40	15	10	Moderate	655	360	14	12
N 112	35	32	25	20	Severe	500	465	23	20
N 448	47	30	10	20	Slight	540	360	12	20
Ca 10	45	50	14	14	Slight	460	355	16	14
Ca 40	55	30	30	13	Severe	435	405	33	30
Ca 80	40	40	10	25	Moderate	550	255	19	30
Ca 240	45	30	20	8	Slight	405	255	22	32
P 0	43	40	30	30	Slight	385	420	10	15
P 0.8	40	40	12	10	Slight	375	365	43	20
P 15.5	23	28	10	10	Moderate	470	420	26	8
P 62	40	35	14	14	Severe	575	280	20	12

* All plants of series C failed to show signs of fluorine injury.

contents of roots and leaves, expressed as parts per million of dry weight, are presented in table III. In addition, by using dry weight values of each tissue, the total fluorine accumulated in the entire plant was calculated, and the percentage of this total present in the leaves was tabulated. In general, plants with the greatest degree of external injury as a result of NaF in the substrate also contained the most fluorine. As shown in table III, the only culture showing fluorine injury in the nitrogen group during the experimental period, 112 p.p.m. N, had a foliage F content of 540 p.p.m. In the plants that were uninjured during this period, the F content of the leaves never exceeded 160 p.p.m. The 14 p.p.m. N plants, being extremely hard, had the lowest absorptive capacity, as illustrated by the comparatively low root and foliage fluorine contents and total fluorine accumulation. Notwithstanding the fact that 33% of the total fluorine absorbed was found in the leaves, this amount was insufficient to cause injury. The 56 p.p.m. N plant had a very high root content of 3010 p.p.m. F but a low foliage content, 96 p.p.m. Probably the medium hard condition of the plant, although allowing for considerable absorption by the roots, inhibited the translocation of fluorine into the leaves, where only 9% of the total fluorine was found. In the 112 p.p.m. N culture there was evidence of rapid translocation as well as absorption of fluorine, resulting in injury to the plant. In the 448 p.p.m. plant, although absorption of fluorine was fairly high, not enough of the total amount accumulated was translocated to the leaves to cause injury.

In the calcium group receiving fluorine through the roots, the F content was highest in the leaves of plants that exhibited symptoms of injury. The foliage of the 40 p.p.m. Ca plants contained 950 p.p.m. F, whereas the foliage of the other plants in this group never exceeded 170 p.p.m. By its tendency to precipitate fluorine, probably in the form of CaF_2, in the nutrient solutions containing high calcium levels, calcium introduced another factor to be considered in interpreting the analytical results. Table III indicates that as calcium was increased in the substrate, a greater amount of fluorine was found in or about the roots. The low percentage of total fluorine found in the leaves of high calcium plants (4 to 9%) suggests that the fluorine was effectively tied up in or around the roots and prevented from entering the translocation stream. In soil studies with tomato and buckwheat, PRINCE *et al.* (**5**) previously reported a reduction of fluorine in the roots and leaves when pH values were raised from 4.5 to 6.5 with calcium.

In the phosphorus group the highest foliage content of 1010 p.p.m. F was found in the plants that were most severely injured. In general, the F content of the foliage increased with an increase in the phosphorus supply. In contrast, the root fluorine content decreased with increase in phosphorus supply. Whereas all of the plants had a fairly high absorptive capacity, increase in phosphorus supply appeared to increase the

mobility of fluorine from roots to leaves, thus causing most severe injury at the highest phosphorus level.

Analytical data from the plants in series B (table IV), which were harvested after different exposures to fluorine, give an indication of the accumulation of fluorine necessary to produce typical fluorine-toxicity symptoms. The F content of the 112 p.p.m. N plants, after 13 days was approximately equal to that of the same plants in series A. The 56 p.p.m. N plants accumulated twice as much fluorine, 1000 p.p.m., over a 24-day exposure before injury appeared on the leaves. The 14 p.p.m. and 448 p.p.m. N plants failed to accumulate sufficient fluorine over a period of 31 days to produce injury.

As in series A, the 40 p.p.m. Ca plants, which showed injury within the shortest time, had the greatest leaf fluorine content. The 80 p.p.m. Ca plants, which were not injured within 13 days in series A, developed injury almost concurrently with the 40 p.p.m. Ca plant, although the fluorine analyses were somewhat lower in the former plant. As in series A, the plants at 10 and 240 p.p.m. Ca accumulated much lower amounts of fluorine in the leaves. Root analyses in this group again indicated that high calcium was effective in preventing the movement of fluorine into the translocation stream.

Plants that received the highest amounts of phosphorus in the substrate and that showed definite signs of fluorine injury within the shortest time, 13 days, also had the highest foliage F content. Root analysis gave further evidence that fluorine in the roots decreases with increased phosphorus supply.

Fluorine analyses of tomato plants exposed to F fumigation

Analyses of all plants in series C (table V), which were fumigated at the lower concentration of .048 p.p.m. F for four and one half hours, showed only a slight accumulation above the normal foliage F content, never exceeding 55 p.p.m. The analytical data bore little relation to the amounts of nitrogen, calcium, or phosphorus supplied to the plants. In general, the fluorine content of the roots was less than half that of the leaves and did not exceed the normal limits for tomato roots, (table IV). The plants harvested seven days after fumigation showed no significant change in F content from those harvested immediately after fumigation.

Series D plants (table V) fumigated at .47 p.p.m. F for three and one half hours failed to indicate as significant a relationship between F content and nutritional status as the plants treated through the roots even though they showed injury symptoms which corresponded to the nutritional treatments. As in the lower fumigation, the F content of the roots was within normal limits, indicating the absence of downward translocation of fluorine from leaves to roots. Analyses of leaves harvested seven days after fumigation were generally lower than those made immediately after fumigation, indicating a loss of fluorine from the foliage during this

time. The question arises regarding the fate of this fluorine lost from the leaves, since there seemed to be no translocation into the roots. Further experiments are in progress to determine whether fluorine is dissipated from the leaves into the surrounding atmosphere (**10**).

Summary

The effects of varied nitrogen, calcium, and phosphorus nutrition on the susceptibility of tomato plants to injury from NaF applications to the roots and HF fumigation of the tops of the plants are summarized as follows:

1. Typical symptoms of fluorine toxicity consisting of tip and marginal scorching of the younger fully expanded leaves were identical whether treatment consisted of fluorine application through the roots or fluorine fumigation of the plant tops.

2. Medium levels of nitrogen, calcium, and phosphorus favored absorption and translocation of fluorine in sufficient quantities to cause visible leaf injury by root treatments and also absorption of toxic quantities of fluorine in plants fumigated at the higher fluorine level. A review of the data suggests that these levels are approximately 112 p.p.m. N, 40 p.p.m. Ca, and 62 p.p.m. P.

3. A low or deficient supply of nitrogen, calcium, and phosphorus to the plant aided in preventing the absorption of toxic amounts of fluorine through the roots or through fumigation. Low phosphorus seemed to have the least inhibitory effect.

4. Excessive amounts of nitrogen and calcium prevented fluorine injury, as did deficiency of these elements. Whether this would also be true of phosphorus if the range were extended high enough to include a luxury supply, or whether phosphorus is actually effective in mobilizing fluorine is not clear.

5. Where calcium was added to the substrate in concentrations above 40 p.p.m., its tendency to precipitate fluorine in the form of insoluble compounds within or around the roots further reduced the possibility of injury to the foliage.

6. Symptoms of severe fluorine toxicity on the leaves were invariably associated with the highest fluorine foliage content in the three nutritional groups in the root fluorine treatment. This was not so marked in the fumigated series.

7. Root injury occurred in the same plants which showed foliage injury in the root-treated series. There was no indication of fluorine injury to roots as a result of fumigation.

8. Root injury was not associated with a high F content, many of the highest fluorine values occurring within the healthiest root systems.

9. When NaF was added to the substrate, although fluorine was translocated to the leaves, the roots almost invariably showed a greater fluorine

accumulation than did the leaves. In contrast, fumigated plants showed a high accumulation of fluorine in the leaves and a normal amount in the roots, suggesting the absence of downward translocation of fluorine from the leaves.

10. The lower fumigation (.048 p.p.m. F) produced no injury on tomato foliage, indicating that this concentration was below the critical rate for injury to tomato. The high fumigation rate (.47 p.p.m. F) produced definite injury.

11. The average fluorine content in the leaves as a result of fumigation at .048 p.p.m. F was 40 p.p.m., whereas at the .47 p.p.m. F fumigation level the average was 477 p.p.m., indicating the probability that, within limits, plants grown under similar environmental conditions absorb fluorine in proportion to the amount present in the atmosphere.

12. Where foliage accumulation of fluorine was brought about by fumigation, there appeared to be a loss in fluorine from the leaf tissue within a seven-day period. No such loss was obvious where fluorine accumulation resulted from fluorine added through the roots.

We are indebted to Dr. W. R. Robbins for his able assistance in the early stages of this investigation.

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SOIL AERATION AS A FACTOR IN WATER ABSORPTION BY THE ROOTS OF TRANSPIRING PLANTS

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(WITH SEVEN FIGURES)

Received August 16, 1949

The relation of soil aeration to water absorption by the roots of intact transpiring plants has been considered by a number of workers whose extensive writings have been the subject of brief reviews (**2, 12, 19, 21**). While a great variety of results and some conflicting data are reported, there is a general agreement that poor aeration decreases the rate of water absorption by plants and may produce wilting when transpiration is sufficiently rapid. In their reviews, KRAMER (**12**) and COMPTON (**2**) suggest that inadequate aeration reduces water absorption as a result either of (1) a decreased permeability of the roots or (2) a decreased absorption surface as a result of the death of rootlets and of the cessation of root growth. Thus aeration may either directly influence the rate of water influx by affecting cellular activities, which control cell permeability, or indirectly affect water absorption as a result of aeration-induced secondary factors.

Separation of the direct and indirect effects of aeration is difficult. The introduction of deficient aeration conditions will, among other changes, produce adjustments in the solute status and metabolic activity of the root cells, affect root growth, and result in the death of some, and ultimately all, of the root tissue. Though these secondary factors cannot be entirely avoided in studies using intact plants, their influence can be minimized by short-time experiments. The differences in root anatomy of water-culture and soil-grown roots (**17**) suggest the desirability of carrying out aeration studies with plants in soil whose moisture content is allowed to vary over the usual field range, i.e., approximately from field capacity to the permanent wilting percentage. However, the use of soil-grown plants in aeration experiments requires that careful attention be given to the effectiveness of aeration control obtained.

It is the purpose of the following experiments to examine the role of soil aeration as a direct factor in passive water absorption by the roots of rapidly transpiring, fairly mature, soil-grown plants under conditions minimizing the influence of unavoidable secondary factors.

Methods

Tomato plants (*Lycopersicon esculentum*, variety Pearson) were grown in Yolo sandy loam in No. 2-size friction-top cans equipped with gas connections in both the lids and the bottoms to permit control of the soil atmosphere around the roots. These plants were grown in a greenhouse, and when approximately 12 inches high, were divided into pairs on the basis of

size and leaf area. Possible differences in the behavior of plants from a given pair were minimized by alternating the treatment given each plant in successive experiments.

The paired plants were allowed to extract the soil moisture to the permanent wilting percentage. When the plants were wilted to an equal degree, the original soil atmosphere surrounding the root systems was displaced by sweeping a gas of known composition through the container. After the soil atmosphere had been replaced, the plants were irrigated with water, which previously had been brought into gaseous equilibrium with the introduced gases. Thus, in addition to the change in soil atmosphere, the roots were bathed by water of known dissolved gas content. Because of the short duration of the experiments, no attempt was made to renew the atmosphere around the roots during the course of each experiment.

The time required by the equally wilted pairs of plants to recover turgor after irrigation under controlled aeration conditions was then observed to obtain a direct measure of the effect of aeration on water absorption by transpiring plants. Recovery time was determined by analysis of successive photographs. The plant was recorded as "recovered" when successive photographs disclosed that the leaflets and petioles made no further adjustment as a result of increasing turgor.

Bringing the plants to the state of wilting characteristic of the permanent wilting percentage has two advantages: (1) a reproducible water deficit is provided, which serves as a common starting point in successive experiments, and (2) the permeability of the soil to gases is markedly increased. A high permeability to gases insures a rapid and essentially complete replacement of the soil atmosphere adjacent to the moist root surfaces. Since the gaseous permeability of soils is greatly decreased when soil-moisture contents are near the field capacity (**5**), it is doubtful whether differential gas treatments carried out in moist soils produce the intended changes in the soil atmosphere near the root surfaces, even though continued for an extended period of time. A complete replacement of the soil atmosphere within the No. 2-size can of sandy loam soil at the wilting point was achieved in five minutes, as indicated by a Pauling Oxygen Analyzer. A photograph of the apparatus used is reproduced as figure 1.

The numbers identify the following portions of the apparatus: (1) Flask for preparing de-aired water under vacuum. (2) Flask containing porous alundum tube through which the test gas bubbles humidifying the gas and saturating the air-free water drawn from flask (1). (3) Burette for measuring gas-saturated water to be used in irrigation of test plant. (4) Flask containing porous alundum tube for humidifying the introduced air. (5) Burette for measuring the air-saturated water for irrigation of check plant. (6) Check plant and test plant in sealed No. 2 cans with lids and bottoms equipped for gas connections. (7) Pressure and vacuum gages to indicate departure from atmospheric pressure in cans and to check for gas tightness of seals. (8) Wet test-gas-meter to record total volume of gas passed through system. (9) Fischer and Porter Flowrater to indicate flow rate of gas sample passed through Pauling Oxygen Analyzer labelled (10).

Nitrogen or carbon dioxide supplied under a small pressure from reducing valves on standard gas cylinders bubbled out through the porous alundum tube in flask (2) [see figure 1], humidifying the gas and saturating the water, which was previously drawn from flask (1), where it had been stored under vacuum to remove dissolved oxygen. The gas then passed out of flask (2), by-passed burette (3), and flowed into can (6) containing the test plant. Air, humidified by bubbling through a porous alundum tube in flask (4), by-passed burette (5), and flowed into can (6) containing the check plant. The importance of any slight difference between the humidity of the input atmosphere and that of the soil atmosphere was minimized by sweeping equal quantities of gas through the cans containing the check plants and those containing the gas-treated plants.

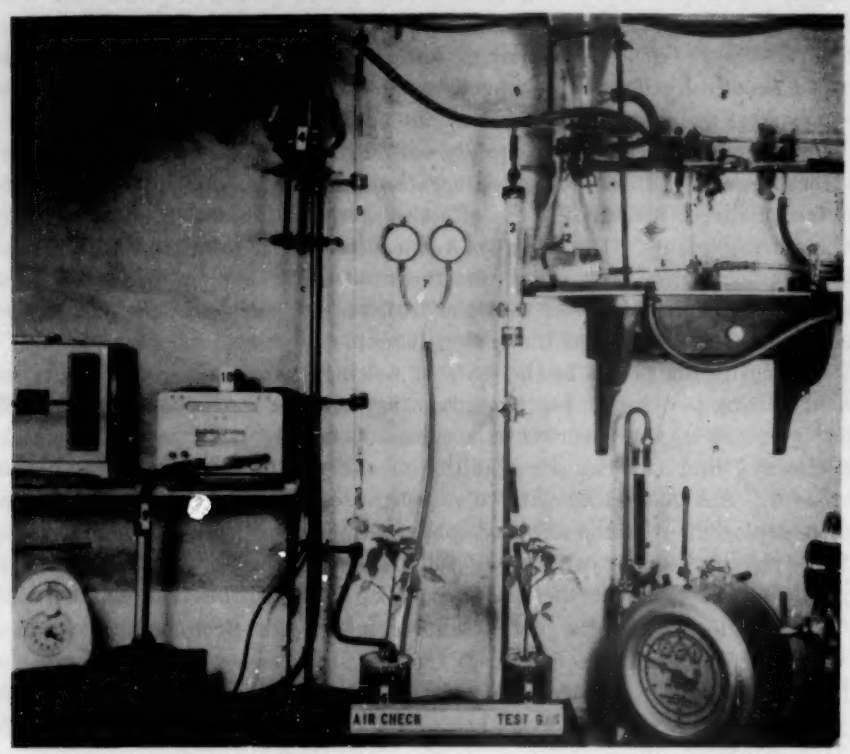


FIG. 1. Apparatus for replacement of soil atmosphere with humidified gas of known composition, and for irrigation with water in equilibrium with the replacing gas.

The gas was drawn out at the bottom of the cans through vents under a slightly reduced pressure provided by a water aspirator and controlled by a pressure regulator consisting of a tube open to the atmosphere and inserted approximately ½ inch below a water surface. This combination of pressure supplied from the gas cylinders and of the reduced pressure from the aspirator permitted a rapid flow of gases through the cans but maintained atmospheric pressure in the cans containing the plant roots. The wet test meter (8) recorded the total volume of gas drawn through the system. The oxygen content of the gas leaving the containers (6) could be periodically or continuously checked with the Pauling Oxygen Analyzer (10). To prevent damage to this instrument, the portion of gas diverted for analysis was kept below 150 ml./min., as indicated by the flowmeter (9). When it was desired to remove traces of oxygen present as an impurity in the tank nitrogen, the nitrogen was passed through two absorption vessels containing alkaline pyrogallol prior to entering flask (2).

Irrigation of the plants in cans (6) was accomplished by manipulation of the two-way stopcocks above and below the burettes (3) and (5). With these stopcocks properly set, gas pressure in flasks (2) and (4) was utilized to fill the burettes. When the burettes were filled to the proper volumes, the upper stopcocks were turned to permit the gases to by-pass the burettes. At the proper time, the lower stopcocks could be turned to permit the gas-saturated water to flow into the cans (6). Thus, it was possible to irrigate the plants with water of known dissolved gas content without admitting external air. As soon as the plants were irrigated, the cans were stoppered at the top and bottom.

All experiments were carried out in a controlled environment room maintained at a temperature of 28° C ± 1° and within a relative humidity range of 35 to 45%. Measurements of the "evaporating power" of the room air using a Livingston white bulb atmometer indicated a mean evaporation rate of 59 ml. of water per day. This evaporation condition approximates that of the outdoor air at Davis, California,[1] during the summer months. The plants were placed on a rotating table and illuminated by a bank of fluorescent tubes, which produced 700–800 foot-candles of light at the tops of the plants. Several oscillating fans kept the air around the plants in constant motion.

Results and discussion

Studies of the time required by wilted plants to recover turgor following irrigation, when carried out on equally wilted plants under constant temperature, humidity, and light conditions, provide a direct measure of the effect of aeration on water movement into transpiring plants under conditions favoring maximum dominance of passive over active water absorption. These measurements can usually be carried out in less than two hours, so that the influence of secondary factors is minimized. This method is simple and graphic. On the other hand, it suffers the disadvantage of permitting one to secure only qualitative or semi-quantitative data because of the difficulties in the exact determination of the time when individual plants recover. By careful standardization of the plants and of the initial degree of wilting and by the use of successive photographs to observe recovered plants, reasonably accurate measurements of the time of recovery are possible. Some typical sets of photographs taken during the recovery periods are reproduced in figures 2 to 7. The accuracy of this work could be increased appreciably by taking photographs more frequently. Nevertheless, the photographs do show distinct differences in recovery time following replacement of the soil atmosphere by nitrogen (see figures 2 to 4), and particularly, by carbon dioxide (see figures 5 and 6). Measurements were taken of the recovery times for the various plant pairs, when both plants were aerated. Photographs made during some of these check experiments are shown in figure 7.

[1] Comparison made with monthly mean readings for the year 1946 as reported by U. S. Weather Bureau Meteorological Station at Davis, California, for the months of May through September, viz., 42.4, 58.8, 64.3, 59.4, and 62.0 ml./day.

The results of these experiments are summarized in table I. The "recovery ratio" used in table I is defined as the ratio of the recovery time of the treated plant to that for the aerated check plant. It is a convenient means for expressing the comparative recovery time of the paired plants.

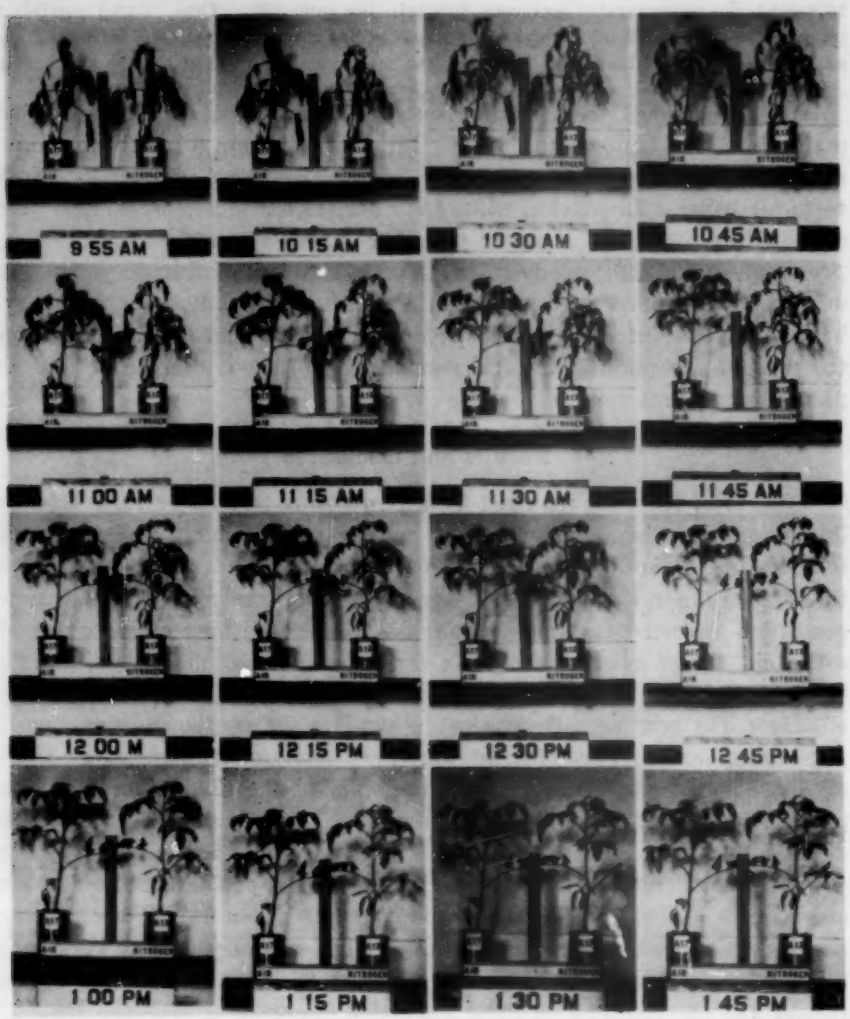


FIG. 2. Run No. 17. Effect of nitrogen saturation of the soil for 12 hours prior to irrigation at 9:55 A.M. Recovery: air at 12:00 A.M., nitrogen at 1:15 P.M. Recovery ratio, 1.6.

The ratios were largely independent of which paired plant served as the aerated check in a given run. Unavoidable differences in the behavior of plants have been compensated by alternating the treatment given individual plants of a given pair in successive experiments. The recovery ratio for the nitrogen-treated plants had a mean value of 1.4 ± 0.08 (S. E. of mean)

and for carbon-dioxide-treated plants, of 2.6 ± 0.17. The greater retardation in the rate of water absorption following saturation of the soil with carbon dioxide must be attributed both to the removal of oxygen and to a direct effect of carbon dioxide on the functioning of the plant. The specific action of carbon dioxide in affecting the passage of water through plant roots has been observed by other investigators (**8, 11**).

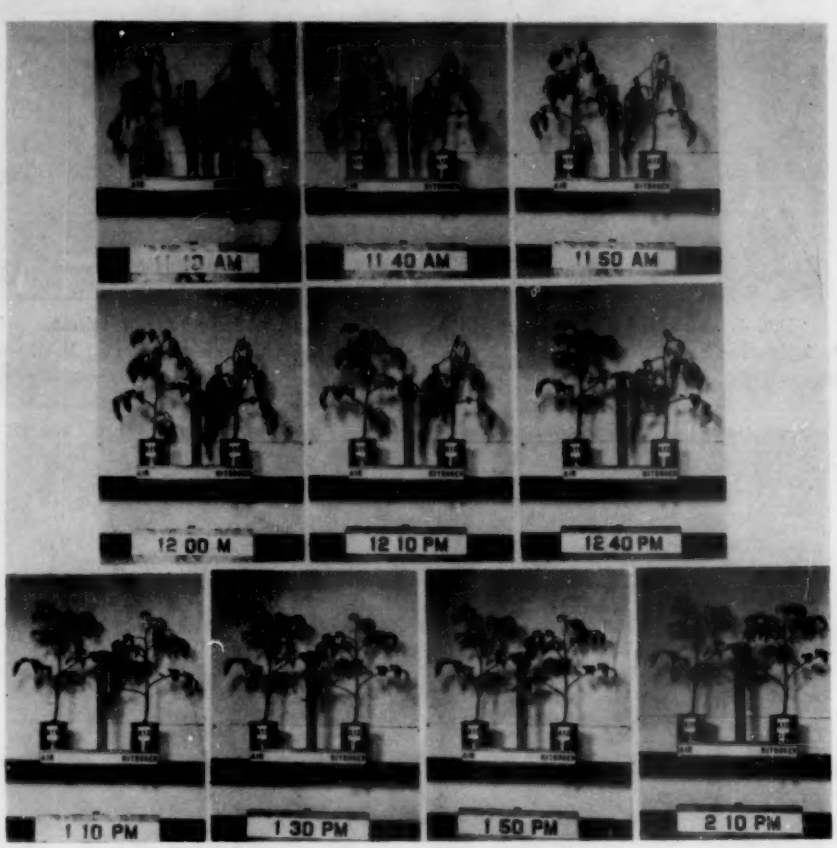


FIG. 3. Run No. 16. Effect of nitrogen saturation of the soil for two hours prior to irrigation at 11:10 A.M. Recovery: air at 12:40 P.M., nitrogen at 2:10 P.M. Recovery ratio, 2.0.

Several factors that might affect the recovery ratios were investigated: namely, (1) the severity of wilting prior to irrigation; (2) the criterion used to select "recovered" plants; and (3) the length of exposure to the introduced gases. Within reasonable limits, the severity of wilting permitted before displacement of the soil atmosphere and the subsequent irrigation was found to have no effect on the recovery ratios, provided that the two plants in a pair were equally wilted initially. The severity of the initial wilt, of course, affected the length of the recovery period. Recovery ratios were found to be unaffected, whether computed at a time of par-

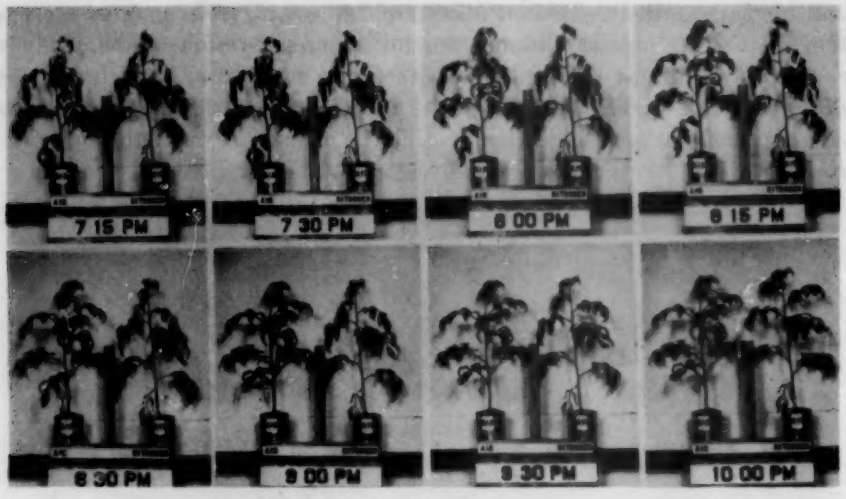


FIG. 4. Run No. 28. Effect of nitrogen saturation of the soil for 10 minutes prior to irrigation at 7: 15 P.M. Recovery: air at 9: 00 P.M., nitrogen at 10: 30 P.M. Recovery ratio, 2.0.

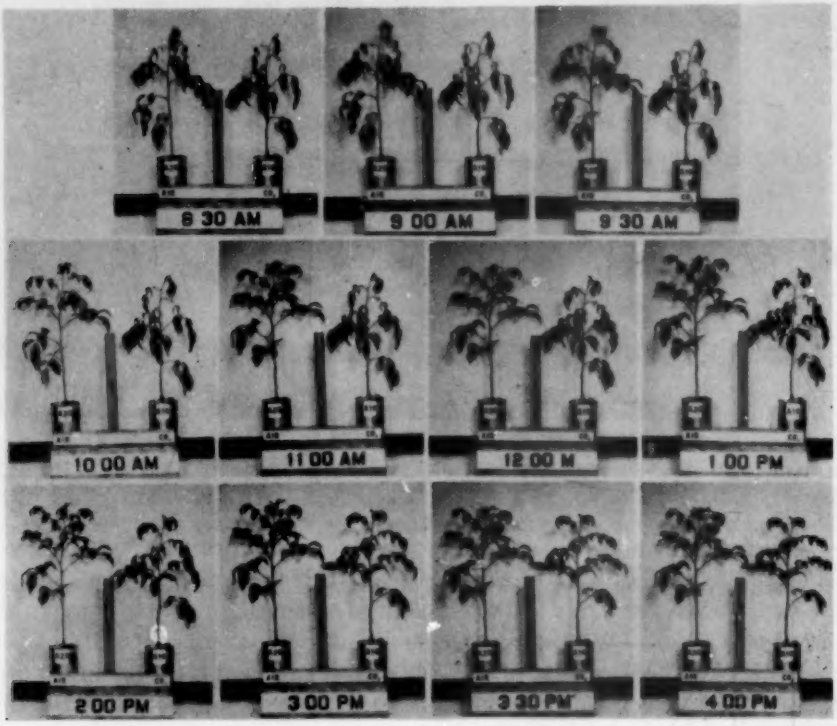
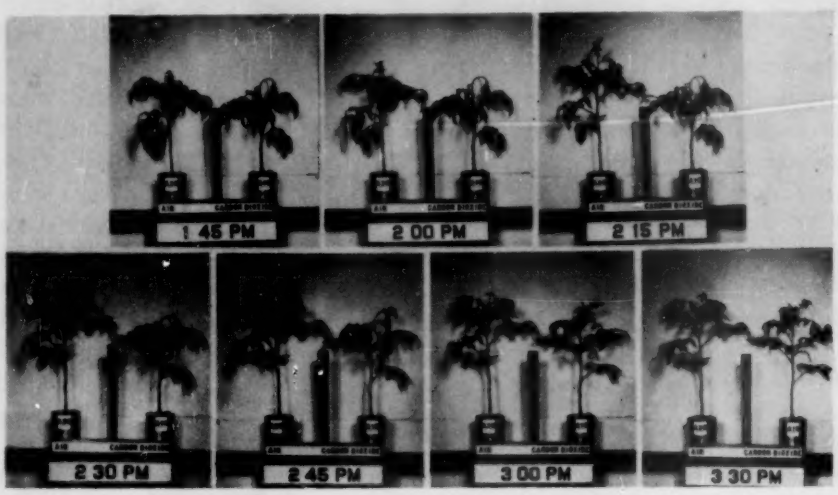
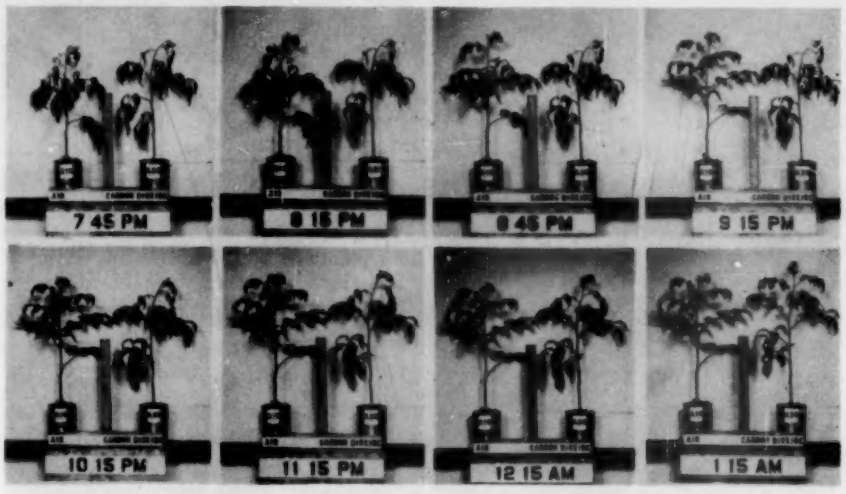


FIG. 5. Run No. 34. Effect of carbon dioxide saturation of the soil for 10 minutes prior to irrigation at 8: 30 A.M. Recovery: air at 11: 00 A.M., carbon dioxide at 4: 00 P.M. Recovery ratio, 3.0.

tial recovery, as determined by measurement of petiole-stem angles, or calculated at a time of apparently complete recovery, as selected by analysis of successive photographs. The data of table I indicate that exposure to



Run No. 26. Recovery: Air at 2: 45 P.M., carbon dioxide at 4: 00 P.M.

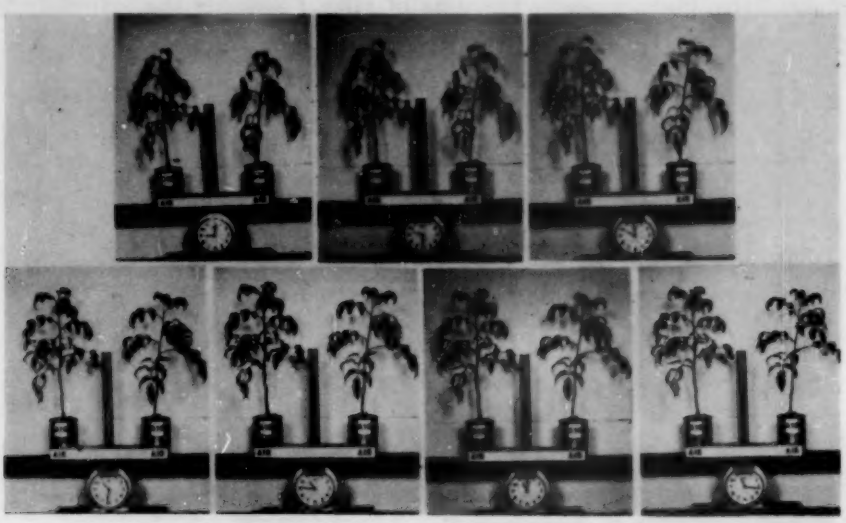


Run No. 30. Recovery: Air at 10:00 P.M., carbon dioxide at 1:45 A.M.

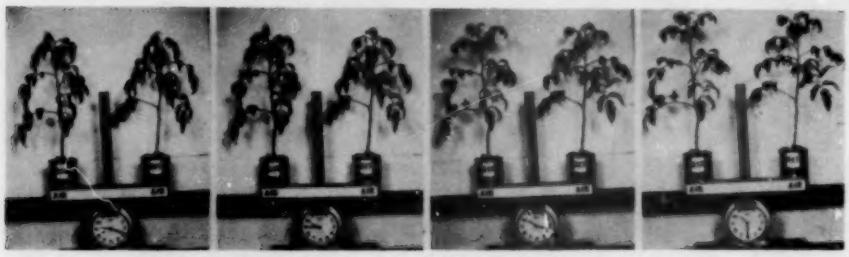
FIG. 6. Effect of carbon dioxide saturation of the soil for 10 minutes prior to irrigation on paired plants. Treatments reversed in successive runs. Recovery ratios: Run No. 26, 2.3; run No. 30, 2.6.

nitrogen for periods up to 12 hours before irrigation is apparently without effect on the recovery ratios. With carbon dioxide, a two-hour prior exposure did not appear to increase the recovery ratio above that resulting from a 10-minute exposure. The effects of longer exposures were not ex-

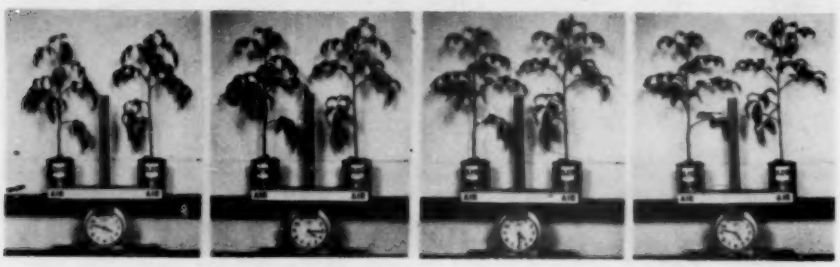
amined, as it was the primary purpose of this study to investigate the short-period effects of controlled soil atmospheres.



Run No. 37B. Recovery: Both plants at 11: 15 P.M.



Run No. 37C. Recovery: Both plants at 10: 30 P.M.



Run No. 37E. Recovery: Both plants at 4: 45 P.M.

FIG. 7. Check runs with the soil around both plants of given pairs aerated prior to irrigation. Recovery ratios for all plant pairs were very closely equal to 1.0.

The recovery of previously wilted nitrogen- or carbon-dioxide-treated plants is perhaps unexpected in view of the observation by HOAGLAND (**7**, p. 116) and others that tomato plants growing in water culture may wilt on

hot days if the aeration is stopped or markedly diminished. From the present experiments, it is evident that even quite severely wilted soil-grown plants, deprived of oxygen or treated with carbon dioxide, will recover turgor within 10 hours after irrigation and generally in less than half this time.

As is well known, the turgor of a plant depends upon the comparative rates of water absorption through the roots and of water loss by transpiration from the aerial portions of the plant, other factors remaining constant and favorable. It follows, obviously, that the wilted plants investigated in the present experiments could recover turgor only if the rate of water absorption, although reduced by the nitrogen and carbon-dioxide treatments, still exceeded the rate of transpiration loss. It is therefore probable that if such experiments were to be carried out under conditions favoring a more rapid transpiration loss, a greater difference in recovery times between the treated and aerated plants would be observed. Probably, if the conditions were sufficiently severe, the nitrogen- and carbon-dioxide-treated plants might fail to show any recovery. Except for the limited light intensity of 700–800 fc, which approximates the intensity of diffuse daylight, the plants in these experiments recovered under transpiration conditions comparable to those often prevailing during the hot summer months at Davis, California.

What explanations can be offered for the observed reduction in the rate of water influx produced by removal of oxygen and for the greater reductions resulting from saturation of the soil with carbon dioxide? The generally accepted need for oxygen in active water influx is not of concern here since, as KRAMER (**12**) states, "Reduction in or cessation of active water absorption cannot explain the large reduction in absorption of (water by) transpiring plants . . . because active absorption can supply only a small percentage (less than 5%) of the water needed by rapidly transpiring plants." Under the conditions of these experiments, which deal with absorption by intact transpiring plants initially under a considerable water deficit, active absorption is probably of even less importance. In their reviews, KRAMER (**12**) and COMPTON (**2**) conclude that reduced absorption by inadequately aerated roots of transpiring plants must result from a decreased absorbing surface or a decreased root permeability.

Let us consider the possibility that poor aeration reduces the rate of water absorption as the result of a decreased absorption surface—a condition caused by a cessation of root growth or by injury or death of absorbing roots.

The initial condition of wilting and the rapidity with which nitrogen and carbon-dioxide treatments affect the absorption of water by these previously wilted plants should exclude cessation of root growth as the causal factor of the reduced rate of water influx resulting from impaired aeration. Where nitrogen was introduced for a period of only 10 minutes before irrigation, a marked difference in rate of recovery between the gas-treated

plant and the aerated check plant was noticed within a short time. In figure 4 this difference is apparent 45 minutes after irrigation, or a total of 55 minutes after the introduction of nitrogen into the system. More striking differences appeared after the carbon-dioxide treatments. In figures 5 and 6, differences in the turgor of the plants may be readily seen after a total exposure of 40 to 60 minutes.

The possibility of injury to the cells in the absorbing regions of the roots caused by the wilting and by the introduction of nitrogen and of carbon dioxide requires consideration. KRAMER (**13**) found that wilting did not cause serious physiological injury, inasmuch as the root systems of wilted plants would exude from cut stumps after irrigation, although they appeared to absorb water more slowly. It is difficult to say whether the reduced rate of water absorption Kramer observed was due (1) to an actual reduction in cell permeability caused, perhaps, by continued suberization of cells in roots that have ceased growth because of water deficit or (2) to a decreased absorbing surface resulting from injury to root tips and root hairs. As for the gases, ROSENE (**18**)[2] found that exposure of turgid roots to nitrogen for four hours produced no permanent injury, and KRAMER (**11**) observed that at least during the first two or three hours the effects of carbon dioxide appeared to be reversible. The roots of wilted plants may be affected even less than turgid roots by the replacement of the surrounding atmosphere with nitrogen or carbon dioxide. When the atmosphere around the roots of the wilted plants was saturated with nitrogen for 10 minutes, the nitrogen then replaced by air, and the plants irrigated with air-saturated water, the mean recovery ratio for two experiments was 1.1. With carbon dioxide, the mean recovery ratio for two experiments was also 1.1. Since these ratios do not differ significantly from 1.0, they suggest that replacement of the atmosphere around the roots with nitrogen or carbon dioxide for a brief period, at least, does not produce a lasting injury that affects water absorption under the conditions of these studies. It appears, therefore, that the reduced rate of passive water absorption observed in the presence of nitrogen or carbon dioxide is not caused by a decreased absorbing root surface but must be the result of a decreased permeability of the root cells.

The suggestion that the effects of aeration on water absorption may involve changes in cytoplasmic permeability has been offered by many investigators, including LIVINGSTON and FREE (**15**), HUNTER and RICH (**10**), KRAMER (**11**), and HOAGLAND and BROYER (**8**). Several of these workers reasoned that absorption was dependent upon permeability of the cell membranes and that the permeability decreased under restricted aeration. Kramer found that water movement through detopped root systems (of sun-

[2] More recently, Miss Rosene in a paper presented before this society at New York, Dec. 1949, reported that, in individual root hairs of *Raphanus sativus*, water influx was reversibly decreased by nitrogen for periods of 90 to 100 minutes, but an anoxic period of 280 minutes produced irreversible injury. (Note added after submission of this manuscript.)

flower and tomato) connected to a vacuum pump was reduced 35 to 56% by exposure to a high concentration of carbon dioxide but was reduced 1 to 13% following introduction of nitrogen. Hoagland and Broyer, using the same technique, found that carbon dioxide and nitrogen cause an initial decrease in the rate of water influx, followed later by an increased influx in the carbon-dioxide-treated plants. The reduced rate of water absorption observed in the present experiments—and by these other workers—following removal of oxygen with nitrogen and carbon dioxide may be conveniently ascribed to a decrease in the effective permeability of the root cells involved in the absorption of water and its transfer into the vascular system of the plant. While this is a handy way to describe the effects of these gases, such a statement gives no clue to the changes within the root that are responsible for the decreased permeability.

The mechanism by which aeration influences the permeability of root cells to the passive transfer of water is a fertile field for study. Several possibilities exist. BLINKS *et al.* (**1**) found that depriving a system of oxygen decreased or abolished the bioelectric potential and that over a considerable period of time the results were reversible. Studies on the electrical properties of protoplasm have been reviewed by LUND (**16**). Changes in cytoplasmic streaming produced by adjustments of the aeration have been observed by a number of investigators, whose work has been reviewed by SEIFRIZ (**20**). In general, carbon dioxide has been found to check streaming quickly. On the other hand, depriving the cells of oxygen by saturation with nitrogen has often failed to stop but has usually retarded streaming. The relation between metabolism and water absorption by plant tissues has been considered by a number of investigators, whose work has been reviewed by CRAFTS *et al.* (**3**) and treated thermodynamically by LEVITT (**14**). In analyzing the evidence linking water absorption and metabolic activity, one must be careful to distinguish the type of water-absorbing system involved in the study reported. For example, ROSENE (**18**), working with non-transpiring systems of excised or intact onion roots, finds that the "velocity of water absorption in roots is dependent on oxygen consumption," while WILSON and KRAMER (**22**), working with transpiring plants, contrary to the earlier conclusions of HENDERSON (**6**), find "no definite relation . . . between the rate of root respiration and the rate of water absorption."

The experiments herein reported indicate that aeration, when limiting, may directly affect the rate of water movement into transpiring plants by controlling cellular activities, which influence root permeability. This direct effect of aeration on passive water absorption appears very rapidly after the roots are deprived of oxygen. Over a longer period of time, poor aeration may further indirectly impair passive water absorption by causing a reduction in root surface, due both to a cessation of root growth and to the death of some of the absorbing roots. The investigations reported in this paper, together with those of KRAMER (**11**), HOAGLAND and BROYER (**8**),

and others, seem to indicate the advisability of amplifying Wilson and Kramer's conclusion referred to above. The following extension is suggested: When the permeability of the root cells is not reduced as a consequence of inadequate aeration, there is no definite relation between the rate of root respiration and that of passive water absorption; but when aeration is deficient, the rate of passive water absorption may be retarded. HOAGLAND (7), writing with reference to the wilting of tomato plants in culture solution following cessation of forced aeration, remarks, "Whether this is merely a matter of permeability changes in root cells . . . is not a finally settled question, but . . . permeability is closely associated with metabolism." An understanding of the mechanism by which aeration of the root environment affects the water relations of plants apparently must await a fundamental study of the physical properties of the cytoplasm, an approach emphasized by HOBER (9).

Summary

Soil aeration as a factor directly influencing passive water absorption by the roots of transpiring, soil-grown tomato plants was studied, using atmospheres of nitrogen and carbon dioxide. Particular attention has been given to obtaining a rapid change of the soil atmosphere.

The effect of nitrogen and of carbon dioxide on the rate of water absorption by the roots of previously wilted, transpiring tomato plants was measured by observing the time required for equally wilted, paired plants to recover turgor following irrigation under controlled soil-atmosphere conditions in a constant-environment room. The ratio of the time of recovery for nitrogen-treated plants to that for the aerated check plants had a mean value of 1.4 ± 0.08 (S. E. of mean) and for carbon-dioxide-treated plants of 2.6 ± 0.17. These ratios confirm observations of others that carbon dioxide has a direct effect on the plant, in addition to that caused by the removal of oxygen. These ratios were independent of the severity of the initial wilting and of the period of gas saturation up to several hours prior to irrigation.

The rate of water absorption decreased rapidly after introduction of the gases. Readily detectable differences in recovery were sometimes apparent after a total exposure of less than one hour, particularly with carbon dioxide. The effects of these gases appear to be reversible over short periods, inasmuch as their replacement by air restored a normal rate of water absorption.

Deficient aeration, presumably by controlling metabolic activities which influence cell permeability, may affect directly and very rapidly the rate of passive water absorption. Over a longer period of time, poor aeration may indirectly retard passive water absorption further by causing a reduction in absorbing root surface.

Acknowledgment is made to Dr. D. I. Arnon, who contributed some of the initial suggestions on the method used.

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BRIEF PAPERS

THE PHOTOINACTIVATION OF ENZYMES BY RIBOFLAVIN

G. R. MANDELS

Received May 19, 1950

Recently GALSTON and BAKER (1) reported that riboflavin could cause the photoinactivation of certain enzymes (urease, tyrosinase, α amylase) by visible light and discussed the significance of this mechanism in interpreting various responses of green plants to light (phototropism, etc.). The present study was undertaken to determine the possible relationship of this type of enzyme inactivation to the physiology of the cellulolytic fungus, *Myrothecium verrucaria,* USDA 1334.2.

Experimental

The susceptibility of invertase to photoinactivation in the presence of riboflavin was demonstrated by exposing a dilute solution of Difco invertase (1:4000) in 0.05 M KH_2PO_4 containing 50 p.p.m. of riboflavin, to the light from a north window. After a two-hour exposure at room temperature an equal volume of 10% sucrose, was added to the exposed solutions as well as to controls maintained in the dark. All of the tubes were then placed in the dark. To determine invertase activity reducing sugars were measured at intervals by the dinitrosalycilic acid method of SUMNER (2). The data (table I) parallel those reported by Galston and Baker.

TABLE I

PHOTOINACTIVATION OF DIFCO INVERTASE BY RIBOFLAVIN

TREATMENT			INVERTASE ACTIVITY*	RELATIVE ACTIVITY
ENZYME	RIBOFLAVIN	LIGHT		
+	+	+	0.1	5
+	−	+	1.6	80
+	+	−	2.0	100
+	−	−	2.0	100
−	+	+	0	0
−	+	−	0	0

* Mg. red. sugar/ml./hr. as glucose.

A similar experiment was carried out in which a suspension of spores was substituted for the Difco invertase in the above experiment. The results (table II) show no effect of light or riboflavin on the invertase of *M. verrucaria* spores. While the concentration of spores was not determined, the suspension used was not sufficiently dense to cut out a large proportion of the incident light.

Another experiment, in which the light exposure was increased to 16 hours of artificial illumination, also failed to show inactivation. The failure to get photoinactivation of invertase extracted from *M. verrucaria*

TABLE II

EFFECT OF RIBOFLAVIN ON INVERTASE ACTIVITY OF *Myrothecium verrucaria* SPORES IN LIGHT AND DARK

TREATMENT		INVERTASE ACTIVITY*	RELATIVE ACTIVITY
RIBOFLAVIN	LIGHT		
+	+	0.26	104
−	+	0.25	100
+	−	0.25	100
−	−	0.25	100

* Mg. red. sugar/ml./hr.

spores is shown in table III. In this experiment lyophilized spores moistened with water were ground with powdered pyrex glass in a mechanical mortar for about two hours, suspended in buffer and centrifuged at high speed and the supernatant decanted through a bacterial filter. While the activity of this preparation was low similar data have been obtained with more active extracts.

TABLE III

EFFECT OF RIBOFLAVIN ON INVERTASE ACTIVITY OF EXTRACTS OF *Myrothecium verrucaria* SPORES

TREATMENT		INVERTASE ACTIVITY*	RELATIVE ACTIVITY
RIBOFLAVIN	LIGHT		
+	+	0.08	94
−	+	0.08	94
+	−	0.085	100
−	−	0.085	100

* Mg. red. sugar/ml./hr.

It was considered possible that some substances in the spores and spore extracts was protecting the invertase from being inactivated by light. If this were so then addition of spore extract to a solution of Difco invertase might protect it from inactivation. In table IV results are given of an experiment to test this hypothesis in which a portion of the same spore extract as used in the preceding experiment (table III) was added. A boiled portion of this extract was added to other tubes. While the data give no indication of any protection this may have been due to a concentration effect—the enzyme activity of the pure invertase being some 30 times that of the spore extract. It is also possible that the two enzymes are different chemically or that the protective substance is coupled with the *M. verrucaria* invertase.

The influence of riboflavin on germination of the spores was determined by inoculating Petri dishes containing agar, salts, 1% glucose and 1% yeast extract at pH 6.5; riboflavin was added at a concentration of 100 p.p.m. to half of the plates which were then placed either in the dark or in

TABLE IV

EFFECT OF SPORE EXTRACT ON PHOTOINACTIVATION OF DIFCO INVERTASE BY RIBOFLAVIN

Treatment			Invertase activity*	Relative activity
Spore extract	Riboflavin	Light		
−	+	+	0.07	5
−	−	+	1.3	87
−	+	−	1.3	87
−	−	−	1.5	100
+	+	+	0.09	6
+	−	+	1.5	100
+	+	−	1.5	100
+	−	−	1.5	100
boiled	+	+	0.06	4
boiled	−	+	1.5	100
boiled	+	−	1.5	100
boiled	−	−	1.5	100

* Mg. red. sugar/ml./hr.

the light of a north window. The riboflavin had no significant effect on germination either in the light or in the dark, approximately 100% germination being observed in all cases after three hours.

To determine whether riboflavin could affect the rate of degradation of cellulose by *M. verrucaria* in the light strips of 8.25 oz. bleached duck (in sextuplicate) were placed on a layer of glass beads in Petri dishes containing inorganic nutrient solution (pH 6.5) to some of which riboflavin was added to give a concentration of 100 p.p.m. The strips were inoculated with *M. verrucaria* spores. One series was placed in the dark in an incubator at 30° C and 80% relative humidity. A second series was exposed to daylight from a north window at room temperature. After seven days the strips were harvested, conditioned, and their breaking strength determined on a motor driven Scott tester. Results (table V) indicate no sig-

TABLE V

EFFECT OF RIBOFLAVIN ON THE BREAKDOWN OF COTTON DUCK IN THE LIGHT AND IN THE DARK BY *M. verrucaria*

Treatment			Breaking strength	Relative* breakdown
Inoculated	Riboflavin	Light		
−	−	−	94 lbs.	
+	+	+	61	89
+	−	+	57	100
+	+	−	32	91
+	−	−	26	100

* As compared to corresponding treatment with riboflavin.

nificant photoinactivation due to riboflavin. The less rapid breakdown in the light is probably due to a temperature effect and to a deficiency of water due to more rapid evaporation from the cultures in the light rather than to a specific effect of light.

Discussion

The data presented here indicate that the enzymes of the fungus *M. verrucaria* are not susceptible to photoinactivation by visible light either in the presence or absence of riboflavin. The implication is that either some protective mechanism is present in this organism or that the specific groups present in certain enzymes which make them susceptible to inactivation by this mechanism are lacking. On the other hand it is possible that the enzyme preparations used by Galston and Baker (alpha-amylase, urease, tyrosinase) contained some photosensitizing substance since the purity of the preparations used was not stated. The data do not permit conclusive evaluation of these hypotheses but they do indicate that the protective mechanism is lacking, since addition of a spore extract to the yeast invertase did not prevent photoinactivation of the latter. It is possible, however, that the concentration of this hypothetical protective mechanism was too low. The difference in behavior of the invertase from yeast and *M. verrucaria* may indicate a more fundamental difference between the two enzymes. Supporting evidence for the latter hypothesis is implied by the slightly different pH optima and the differences in Michaelis' constants (**3**).

Summary

It was found that while riboflavin caused rapid photoinactivation of a purified invertase preparation from yeast (Difco analytical) no effect was found on the invertase activity of *M. verrucaria* spores, nor upon the invertase activity of extracts of ground spores. Riboflavin in the light had no inhibitory effect on the germination of the spores and no effect upon the breakdown of cellulose by this organism.

Grateful acknowledgment is due Miss Anna B. Norton for her technical assistance and to Drs. A. W. Galston and R. G. H. Siu for their helpful comments and discussion.

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TECHNIQUE FOR GROWING PLANTS WITH ROOTS IN A STERILE MEDIUM

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(WITH ONE FIGURE)

Received March 20, 1950

In order to test the effect of adding various organic materials to the mineral nutrient medium of *Phaseolus lunatus* (Burpee's Bush Lima Beans), it was felt desirable to develop a technique for growing them in such a way that the root system would be in a sterile medium. Growth of lower plants (fungi and algae) in such a medium is standard procedure. Moreover, some such work with higher plants has been done (**5**). Whole tobacco plants, for example, have been grown aseptically in Erlenmeyer flasks (**4**). The maturity to which a plant can be raised in this way is limited by its size. A possible solution for this is to grow the root aseptically but with the stem and leaves in the air. This was done in one way by KNUDSON and SMITH (**1**). However, their method required transfer of the seedling plants from their germinating container to another container for further growth. The method described below avoids this complication by disinfecting the seed and planting it aseptically into the top of a specially designed jar. Upon germination the root passed down into the jar and continued its development in the sterile medium found there.

Seed disinfection

Disinfecting was done with bleach solutions made with HTH (high test hypochlorite), according to the method described by WILSON (**6**). The procedure is to take 10 grams of the powder, slurry it in 140 ml. water, and let it stand for 10 minutes. The clear liquid is then decanted and poured over the beans. Wilson used commercial chloride of lime with 28% available chlorine, while HTH has 48% available chlorine. A 1:1 water dilution of Chlorox (Chlorox Chemical Co., 5.25% by weight sodium hypochlorite) will work satisfactorily.

Construction of jar units

The jars themselves were of glass and were 9 cm. tall, 9 cm. in diameter, and had 9 cm. mouths. A hole 3.5 cm. in diameter was punched in the center of each metal, screw-type lid. Into it was inserted a 3.5 cm. long cylinder of thin-walled aluminum tubing, the lower end of which had been turned down on a lathe to leave a shoulder. The thin edge was then flanged down, clamping the lid between it and the shoulder. A disc of thin aluminum sheet was cut to fit the inside of each lid and punched at the center with a hole 3.5 cm. in diameter.

[1] Lederle Fellow in Biophysics.

The entire unit was assembled as follows: A piece of cotton gauze (28 × 24 mesh) was laid over the hole of an inverted lid. On top of this an aluminum disc was placed. The lid was screwed into place on a jar containing any desired nutrient solution. Next, the cylinder was half-filled with Terra-Lite (Zonolite Co., vermiculite) and a gauze-wrapped cotton plug was inserted in its top. This entire unit was autoclaved 45 minutes at 15 lb. steam pressure. After cooling, it was ready for the planting operation.

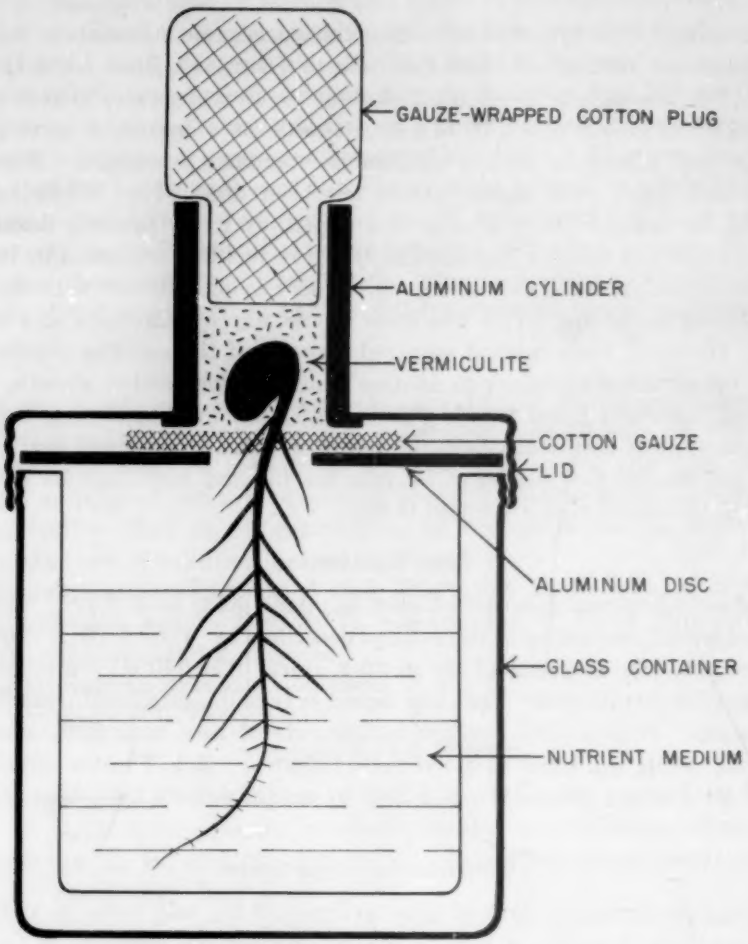

FIG. 1. Cross-sectional diagram of jar unit.

Planting procedure

After the beans had soaked 30 minutes in the bleach solution previously described, they were given five rinses with sterile, distilled water. Into the vermiculite in each aluminum cylinder one of these beans was transferred aseptically. They were then watered with sterile, distilled water. The top

of the cylinder was flamed and then reclosed with the plug. Four days later the beans had germinated sufficiently so that the root had penetrated downward through the vermiculite, the supporting gauze, and the two cm. air space into the nutrient solution (fig. 1). Soon after this the plumule emerged from the top of the cylinder, pushing out the plug. The stem was then wrapped with a pad of cotton-filled gauze; this was pushed down into the top of the cylinder to help support the plant. As a test of technique a nutrient medium consisting of 2% sucrose, 0.01% Difco yeast extract, and 0.01% casein hydrolysate (General Biochemicals, Inc., "Vitamin Free") in Shive's R5S2 mineral nutrient solution (**2, 3**) was used. Plants grown this way developed to heights of 20 to 30 cm. Contamination generally occurred in less than one jar in 20.

Summary

A simplified technique for growing plants with their roots in a sterile medium has been developed. Lima beans (*Phaseolus lunatus*) were treated with a bleach solution, then planted aseptically into the top of a specially designed jar unit. The germinating bean forced its root down into the sterile medium in the jar, while the top of the plant pushed itself out into the air. This procedure avoided transfer of the young seedling from a germination container to a growth container. The method may be used for experiments in which it is desired to test the effects of various substances in the nutrient solution which would be conducive to bacterial or fungal contamination.

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NOTES

Dr. Walter Thomas' Seventieth Anniversary.—The American Society of Plant Physiologists is pleased to felicitate Dr. Thomas on the occasion of his 70th anniversary and to express its appreciation for his many years of service to it and to the profession. Walter Thomas was born on December 3, 1880, at Swansea, a coast town in southern Wales, youngest of four children of David Henry and Mary T. Thomas. His father was a wholesale woolen merchant, but after retirement served as a minister in the Baptist church. Walter's boyhood and young manhood, to the completion of his Bachelor of Science degree was spent in Wales, where he attended in succession the public elementary schools and the Technical College of Swansea, from which he was graduated in 1900. He received the Bachelor of Science degree in 1905 from the University of Wales.

Dr. Thomas' professional career has been the following: Assistant in Research, University College of Wales, 1905–1906; Assistant Research Chemist, Wellcome Chemical Laboratories, London, 1906–1907; Chemist, Canada Department of Agriculture, 1908–1909; Associate Chemist, The Pennsylvania State College Agricultural Experiment Station, 1910–1915; British Chemical Warfare Service, 1915–1917; Assistant Professor of Agricultural Chemistry, The Pennsylvania State College, 1917–1922; Associate Professor, 1922–1927; Professor of Phytochemistry, 1927–1937; Professor of Plant Nutrition, Department of Horticulture, 1937 to the present.

Dr. Thomas is a member of the following professional societies: American Society of Plant Physiologists, American Society of Agronomy, American Chemical Society, American Society for Horticultural Science, Soil Science Society of America, American Association for the Advancement of Science, and Pennsylvania Academy of Science. He has been active in each of these, and has contributed numerous articles to their journals. His honors include the Sc.D. (honorary) degree from the University of Wales in 1928; membership in the Court of Governors, University College of Wales, since 1935; Fellow of the American Association for the Advancement of Science; the Charles Reid Barnes Award of Life Membership in the American Society of Plant Physiologists (1943), of which he is a charter member and which he has served on the Finance Committee since its organization; membership in Sigma Xi, Phi Kappa Phi, Phi Lambda Upsilon, and Gamma Sigma Delta. He became an American citizen in 1930.

His published research papers number approximately 80, and several now are in the press. These cover methods of carbohydrate analysis, carbohydrate and nitrogenous metabolism of plants, ultimate analysis of soils, leaf analysis, and as his major contribution to plant physiology a series of articles on foliar diagnosis as a method for determining the mineral nutrient requirements of plants and the relations between mineral nutrition and the growth and development of crops. In addition, he has contributed abstracts

of articles on plant physiology and chemistry to Chemical Abstracts and Biological Abstracts during many years.

Most of Dr. Thomas' work has been research, and only in recent years has he devoted any of his energies to teaching. His influence in the field of the mineral nutrition of plants is world-wide, through both his publications and a very extensive correspondence with plant physiologists throughout the world. His interests are not limited to plant science, however; he is a true liberal, to whom nothing humane is alien.

Annual Report of the Executive Secretary-Treasurer of the American Society of Plant Physiologists for 1949–50. —Professor Stanfield's report from July 1, 1949 to June 30, 1950 shows steady gains by the A.S.P.P. in membership, subscribers and finances.

Membership: Active members, July 1, 1949, 810; Renewals, 8; New members, 99; Resigned, dropped, deceased, 32; Active members, July 1, 1950, 885.

Subscriptions: Volume 24 in U.S., 349; Foreign subscriptions, 476; Total for Volumes 24, 825. Volume 25 in U.S., 351; Foreign, 488; Total for Volume 25, 839.

Receipts:		Disbursements:	
Dues	$ 4,246.95	Printing and Mailing PLANT PHYSIOLOGY	$12,480.63
Sales and Subscriptions	9,642.93	Editorial Expense	437.91
Editor	107.20	Executive Secretary-Treasurer and Secretary Expense	400.98
Interest	640.22	Convention and A.I.B.S. dues	872.47
Gift	200.00	Refunds and Back Numbers Bought	274.67
Reprints and Overrun charges	2,678.74	Office Supplies, Printing, Postage	252.66
Monograph	25.00		
Total Receipts	$17,541.04	Total Disbursements	$14,719.34

Excess Receipts over Disbursements	$ 2,821.70
Opening Cash Balance	10,657.71
Final Cash Balance	$13,479.41
Securities	18,400.00
Assets of Society	$31,779.41

The Society is fortunate in being able to maintain a sound financial condition despite greatly increased costs of printing its journal and conducting its annual meetings. The officers solicit the continued support of members in encouraging associates and young plant physiologists to join the society. The secretaries will be pleased to send membership application blanks upon request or to contact prospective members whose names and addresses are submitted.

Annual Review of Plant Physiology—Volumes I and II. D. I. ARNON, Editor. Annual Reviews Incorporated, Stanford, California. 364 pages, 1950. $6.00.

This volume includes: Mineral Nutrition of Plants, E. G. Mulder; Carbon Dioxide Fixation by Green Plants, A. A. Benson and M. Calvin; Influence of Light on Plant Growth, M. W. Parker and H. A. Borthwick; Plant Tropisms, A. R. Schrank; Organic Acid Metabolism, K. V. Thimann and W. D. Bonner, Jr.; Transformation of Sugars in Plants, W. Z. Hassid and E. W. Putman; Growth Regulating Substances in Horticulture, J. W. Mitchell and P. C. Marth; Herbicides, A. G. Norman, C. E. Minarik, and R. L. Weintraub; Physiology of Cell Wall Growth, A. Frey-Wyssling; Postharvest Physiology and Biochemistry of Fruits, J. B. Biale; Respiration of Higher Plants, D. R. Goddard and B. J. D. Meeuse; The Nitrogenous Constituents of Plants with Special Reference to Chromatographic Methods, F. C. Steward and J. F. Thompson; Water Relations of Plant Cells and Tissues, P. J. Kramer and H. B. Currier; Soil Moisture in Relation to Plant Growth, F. J. Veihmeyer and A. H. Hendrickson; Soil Chemistry in Relation to Inorganic Nutrition of Plants, P. R. Stout and R. Overstreet.

Volume II of this series will contain: Mechanisms of Absorption and Transport of Inorganic Nutrients in Plants, R. N. Robertson; The Role of Inorganic Nutrients in Plant Metabolism, E. J. Hewitt; Photochemical Reactions in Photosynthesis, J. Franck; Carbon Path in Photosynthesis, H. Gaffron and E. W. Fager; Biogenesis of Chlorophyll and Related Pigments, S. Granick; Respiration of Plants, J. S. Turner; Dormancy and Vernalization, F. G. Gregory; Formation, Occurrence, and Breakdown of Growth Substance in Plants, P. Larsen; Herbicides, G. E. Blackman and W. G. Templeman; Nutritional Requirements of Isolated Plant Tissues and Organs, P. R. White; Frost and Drouth Resistance in Plants, J. Levitt; Physiological Aspects of Low Temperature Preservation of Plant Products, M. Joslyn; Experimental Morphogenesis, R. H. Wetmore; and Gas Damage to Plants, M. D. Thomas.

Annual Review of Biochemistry, Volume XIX.—Edited by J. MURRAY LUCK. Annual Reviews, Incorporated, Stanford, California. 596 pages, 1950. $6.00.

This volume contains twenty-three sections of which the following will be of special interest to plant scientists: Biological Oxidations by V. R.

Potter; Proteolytic Enzymes by M. Laskowski; Nonoxidative, Nonproteolytic Enzymes by P. P. Cohen and R. W. McGilvery; Carbohydrate Chemistry by M. L. Wolfrom and J. M. Sugihara; Chemistry of Lipids by H. J. Deuel, Jr.; Chemistry of Amino Acids and Proteins by R. K. Cannan and M. Levy; Nucleic Acids, Purines, and Pyrimidines by G. Schmidt; Carbohydrate Metabolism by S. Ratner and E. Racker; Metabolism of Proteins and Amino Acids by P. P. Swanson and H. E. Clark; Water-Soluble Vitamins by E. E. Snell and L. D. Wright; Nutrition by H. M. Sinclair; Pyrrole Pigments by R. Lemberg and J. W. Legge; Biochemistry of Antibiotics by H. E. Carter and J. H. Ford; Partition Chromatography by A. J. P. Martin. The volume contains comprehensive subject and author indices.

Biochemie und Physiologie der Sekundären Pflanzenstoffe. Part 2 of Volume I.—KARL PAECH. Springer-Verlay, Reichpietschufer 20, Berlin W 35, Germany, 268 pages. Price 27 German marks; Paper binding, 24 marks.

This book provides an organized, systematic, and critical compilation of existing recent information on the biochemistry and physiology of the so-called secondary plant constituents. The author has endeavored to make the subject matter of interest and value to chemists, biological scientists, technologists and agriculturalists. The book consists of eight sections as follows: General types of metabolism; The lower carbon-acids, Fat metabolism of plants; Terpene compounds; Nitrogen-free aromatic compounds; Secondary plant compounds containing nitrogen, amino acid derivates, Summary. Each of these sections contains several subdivisions grouped primarily on the basis of different compounds and in most instances also a discussion of the physiology of the various substances, the biochemistry of which has been presented. Over 600 citations to recent literature are given as well as a complete subject index.

Chemistry and Industry of Starch.—RALPH W. KERR, Editor. Second Edition. Academic Press, Incorporated, 125 East 23rd Street, New York, New York. 719 pages, 1950. $11.50.

This volume brings up to date our accumulated knowledge of starch and of the more important products derived therefrom. Discussions on the technology of starch have been extended in this second edition. The chapter on the manufacture of corn starch has been rewritten and a chapter on miscellaneous uses of starch which have become important in recent years has been added. Also a chapter on analytical methods most commonly used with starch or starch products has been added. The co-authors of the book include G. V. Caesar, L. M. Christensen, E. F. Degering, Dexter French, Alexander Frieden, Eric Kneen, J. M. Newton, George Peckham, Jr., and G. M. Severson. The volume is divided into five sections which are: Occurrence in Nature; Preparation; Properties; Reactions; and Uses and Analysis. It also includes extensive author and subject indices.

An Introduction to Plant Physiology.—O. F. CURTIS and D. G. CLARK. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York 18, New York. 752 pages, 1950. $6.50.

This textbook is intended for the relatively large number of beginning students in plant physiology who have not had a course in organic chemistry. The biochemical aspects are treated somewhat more briefly than in many textbooks, but other phases of physiology are developed in considerable detail. Water relations and ecology as they relate to horticultural and agronomic practice are quite comprehensively presented. Recent advances in all phases of physiology, such as hormones and the use of hormone-like weed killers, respiration concepts, and radioactive tracer element techniques are included. There is unique treatment of frost hardening, death by freezing or desiccation, root growth, salt antagonism, and factors affecting food translocation. One controversial topic, the translocation of solutes, is discussed at length since one of the authors (the late Professor Curtis) carried out many investigations of this process and his contributions are given in detail. The authors also present critical evidence on several other controversial topics. The book embodies the long and successful teaching experience of the authors especially of students who do not possess the extensive background in chemistry, physics and mathematics presupposed in many similar textbooks. The originality of organization and mode of presentation of subject matter distinguish this textbook from others on the subject of plant physiology. Teachers and students will welcome these features and find the emphasis on evaluation of data particularly useful. Throughout the book specific attention is given to the scientific method of investigation and interpretation of plant processes.

Each chapter has citations to the literature and concludes with questions and problems which emphasize the application and relationship of observations and principles in their solution. A special appendix at the end of the book carries the answers to the questions of each chapter. Two helpful appendixes on False or Unsatisfactory Statements and on Fallacious Reasoning are included to provide examples of common mistakes for beginning students. An index by author and another by subject are included.

Who Knows and What: A new A. N. Marquis Company publication. Selected scientists have received, or shortly will receive, from The A. N. Marquis Company requests for information pertaining to their personal specialties within their occupational fields. Information submitted will be reviewed to determine suitability for inclusion in the company's new and unique reference work, WHO KNOWS—AND WHAT, the Second Edition of which has been put into immediate compilation following the widespread acceptance of the First Edition, published earlier this year.

The unique reference value of WHO KNOWS stems, according to the publishers, from two major features: 1) the inclusion of only those persons having special skills or knowledge in one or more of thousands of highly

specific topics judged by the editors to be subject to general reference interest, together with information on research and experience in these fields; general public or professional eminence is not a deciding factor in selections. 2) the Locator Index, which refers the user by means of a simple key to the listing of any person in the book having special knowledge about any one of the subjects included in the Index.

The company, which also publishes WHO'S WHO IN AMERICA, will welcome any suggestions from A.S.P.P. members and users of Marquis Company publications. Communicate directly with the company at 210 E. Ohio Street, Chicago 11.

Association of Southern Agricultural Workers.—The Plant Physiology Section of this group has designated the following officers for 1950–51: Chairman, HENRY C. HARRIS, Florida Agricultural Experiment Station; Vice-Chairman, G. M. SHEAR, Virginia Agricultural Experiment Station; Secretary, HOWARD B. SPRAGUE, Texas Research Foundation; Directors, L. L. DANIELSON, Virginia Truck Experiment Station, E. M. EMMERT, University of Kentucky, and S. F. THORNTON, F. S. Royster Company, Norfolk, Virginia.

This section will have a program of papers at the annual meeting of the Association of Southern Agricultural Workers to be held at Memphis, Tennessee, February 5, 6, and 7, 1951. Further details on the program will be announced later. Titles of papers for presentation at this meeting will be accepted by the chairman or the secretary of the section.

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